# SEA-TO-LAND NUTRIENT TRANSFER BY SEALS AND SEABIRDS ON SABLE ISLAND:

## Isoscapes Revealed by Stable Isotope Analysis of Vegetation With an Echo in the Island's Feral Horses

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# ABSTRACT

Recent research using stable isotope analysis has shown a dependence on migrating or breeding populations of vertebrates as vectors for the transfer of marine-derived nutrients within coastal ecosystems. Sable Island, Nova Scotia, Canada supports numerous species of plants, a variety of seabird colonies (including common [*Sterna hirundo*] and Arctic [*Sterna paradisaea*] terns), the world's largest grey seal (*Halichoerus grypus*) breeding colony, and a self-sustaining population of wild (feral) horses (*Equus ferus caballus*). I hypothesize that nitrogen cycling within this island ecosystem is highly influenced by the input of nutrients from seals and seabirds ('biogenic vectors'), affecting primary production and potentially stabilizing higher trophic levels (i.e., horses). To examine this relation I developed a spatially-explicit isoscape for Sable Island through stable isotope analysis of nitrogen ($\delta^{15}N$) in samples ($n$ = 282) of marram grass (*Ammophila breviligulata*). I incorporated significant variables (i.e., distance to vector colony and distance to shoreline, $r^2 = 0.41$) into the final parsimonious interpolation model using universal co-kriging techniques. The greatest $^{15}N$ enrichment occurred within the tips and along the perimeter of the island, coinciding with greater densities of grey seals, while the lowest values occurred within the centre of the island.

I then identified individual contributions of seal-, tern- and horse-mediated transfer of marine-derived nutrients inland. Marram grass exhibited higher $\delta^{15}N$ within seal ($\mu$ = 7.5‰) and tern ($\mu$ = 5‰) colonies, while horses and biogeochemical processes (i.e., volatilization, ammonification, etc.) most likely contributed to the homogeneity within the centre of the island ($\mu$ = 3.6‰). Due to the higher densities, wider distribution, and greater $^{15}N$ enrichment of marram tissues, grey seals appear to be the most important vector species while seabirds have a more localized effect. The greater availability of N within vector colonies supplemented the local vegetation community, contributing to greater vegetation cover within colony boundaries. This relation had secondary effects on the horse population, which showed correspondingly higher horse $\delta^{15}N$ values within the tips of the island ($\delta^{15}N$ + 1.6‰) due to consumption of $^{15}N$ enriched forage. I conclude that biogenic vector species promote nutrient transfer by establishing nutrient gateways which indirectly cause cascading effects throughout the food web.

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‰: Per mil (parts per thousand)

$\delta^{13}C$: Difference ratio of $^{13}C/^{12}C$

$\delta^{15}N$: Difference ratio of $^{15}N/^{14}N$

GIS: Geographic information system

GLM: Generalized linear model

GPS: Global positioning system

P/A: Perimeter to area ratio

PRS: Plant root simulator

RMS: Root mean square

TN: Total nitrogen

# 1 INTRODUCTION

The availability of nutrients (i.e., nitrogen and carbon) depends on ecological cycles that sequester or restore nutrients through both abiotic and biotic processes. Nutrient cycling and transfer in terrestrial, freshwater, and coastal systems have been well examined; however, studies have been limited to examining localized effects due to an increase in the complexity and predictability of models at higher scales (i.e., landscape, regional, etc.) (Falkowski et al. 2000; Vanni 2002; Gruber and Galloway 2008). Nitrogen (N) and carbon (C) have been shown to be both from autochthonous (i.e., from intrinsic sources) and allochthonous sources, but the relative importance of each is not well understood (Huxel et al. 2002; Huiskes et al 2006). Allochthonous inputs encompass the movement of organic compounds that are fixed in one habitat to another (Huxel et al. 2002). This includes the movement of detrital inputs (i.e., leaf litter into soil systems), the transfer of greater productivity (i.e., transitioning productivity from pelagic to benthic zones) and the movement of prey species (Polis and Hurd 1996; Huxel et al. 2002). Sandy-island ecosystems provide a useful template in understanding nutrient cycling since the majority of nutrients are allochthonous (i.e., from extrinsic sources) (Huiskes et al. 2006). This may be attributed to sandy-islands exhibiting a continual loss of essential nutrients through leaching and oceanic washouts.

Resource availability has been shown to govern a community's productivity; therefore, nutritional input from extrinsic sources could increase local resource availability above its intrinsic capacity (Sanchez-Pinero and Polis 2000). Abiotic allochthonous inputs of nutrients occur through precipitation, fog, sea-originating-detritus and sea spray; all of which occur commonly on island ecosystems (Clayton 1972). Islands possessing additional nutrient supplementation from biogenic sources (i.e., migratory animals, detritus, etc.) sustain higher nutrient concentrations for longer periods and display greater spatial variability (Bokhorst et al. 2007). Previous studies on islands have examined the importance of perimeter-area ratios (P/A), indicating increased transfer with an increase in the P/A ratio (Anderson and Wait 2001; Stapp and Polis 2003). This relation is true for abiotic transfer, but not as important for biotic vectors since their permeability depends on the physical limitations presenting by the landscape (Anderson and Polis 1999).

The importance of allochthonous nutritional supplements by vector species should be examined in order to determine the direct and indirect effects vector species have on local community stability and structure. To determine the presence of allochthonous nutrients via biogenic vector species, stable isotopes (i.e., nitrogen $\delta^{15}N$ and carbon $\delta^{13}C$) can be used to examine the spatiotemporal variability of nutrients across the landscape (i.e., by constructing an isoscape [Bowen 2010]).

## 1.1 ISOTOPES AND ISOSCAPES

Stable isotopes have been extensively used to better understand ecological interactions including trophic dynamics, environmental interactions and determining source origins of nutrients (Petterson and Fry 1987). Natural isotopes can be used as elemental tracers through the recognition of their predictable changes within an ecosystem (Huiskes et al. 2006). Naturally occurring ratios ($\delta^{15}N$ and $\delta^{13}C$) of $^{15}N$ (0.4%) to $^{14}N$ (99.6%) as well as $^{13}C$ (1.1%) to $^{12}C$ (98.9%) display variability within biological systems and their encompassing species (Farquhar et al. 1989). This is primarily due to the combination of two processes, mixing and fractionation, which combine and separate isotopes, respectively. Isotopic mixing acts to blend multiple sources into a homogenous value within the tissue sampled, while fractionation involves the differential separation of isotopic pools into heterogeneous values through discrimination (Peterson and Fry 1987; Schoeller 1999). The combination of these two processes not only creates isotopic variability among ecosystems and the species that encompass them, but provides characteristic isotopic signatures that can be used to examine differential uptake of nutrients within an ecosystem (Hobson et al. 1996).

Both $\delta^{15}N$ and $\delta^{13}C$ have been used to examine the contribution of marine-derived nutrients to terrestrial communities in a variety of landscapes (Mizutani and Wada 1988; Erskine et al. 1998; Farina et al. 2003). These studies indicate a positive relation between the density of biogenic vectors (i.e., largest isotope enrichment) and higher $\delta^{15}N$ within plants and soils. $^{13}C$ has been more applicable in studying consumer diets where $\delta^{13}C$ values are indicative of the average time-integrated diet of a consumer (DeNiro and Epstein 1978; Ramsey and Hobson

1991). Using $\delta^{13}C$ in studies examining plant uptake of marine-derived C is not efficient since plant photosynthetic gas exchange and $CO_2$ intake from the surrounding atmosphere causes leaf $\delta^{13}C$ values to resemble atmospheric $\delta^{13}C$ rather than marine-derived C (Ehleringer and Cerling 1995). Therefore, local variabilities in plant $\delta^{13}C$ are attributed to differences in photosynthetic water-use efficiency, environmental gradients affecting water-use (i.e., altitude, temperature, etc.) and plant physiology (i.e., stomatal conductance, leaf/root ratio, etc.) (Farquhar and Richards 1984; Ehleringer and Cerling 1995; Panek and Waring 1995; Sah and Brumme 2003). Although plant $\delta^{13}C$ values show little difference between areas with marine-derived nutrient supplementation and those without, they can be useful in comparing landscape $\delta^{13}C$ values between sites (Cocks et al. 1998; Farina et al. 2003).

Derived from the joint-term "isotope-landscape", an isoscape is the spatial and temporal distribution of stable isotopes across a specific landscape (Bowen 2010). The incorporation of process-level isotope distribution models into landscape Geographic Information System (GIS) maps provide an avenue for predicting spatially explicit isotopic ratios. In basic form, isoscapes are prediction models that describe the spatial patterning of isotopes by using known locations and applying probability functions to determine isotopic values at unknown locations. Isoscapes can be used to identify sources, understand nutrient fluxes or storing processes, examine nutrient partitioning and in identifying spatiotemporal movement within biological communities (Dawson et al. 2002; Huxel et al. 2002; Lott and Smith 2006; Hobson et al. 2010). Tracing wildlife movements across a landscape using naturally occurring isotopes can be achieved by linking known isotopic signatures of a species with corresponding isotopes within local plant communities (Paxton et al. 2007; Jaeger et al. 2010). This process can be inconsistent due to the lack of predictability and numerous source effects governing isotope variability (e.g., fractionation rates, spatial heterogeneity, etc.). Interpolation-models can be used to estimate the importance of source origins by examining the source's positive or negative effects on the variable; however, sources must possess verifiably distinct isotope signatures in order to identify their individual effects within an ecosystem. If spatial variabilities exist in the distribution of isotopes, then the interpolation model can be used to determine relations between differing sources and nutrient levels within the local environment (i.e., plant community, higher consumers, etc.). This can help researchers understand local trophic dynamics as a function of

resource availability. Point-sourced nutrient mapping of a relatively isolated system (i.e., islands) will lead to a more functional isoscape model, which in turn may lead to a more accurate understanding of trophic relations within the community.

## 1.2 NITROGEN AND CARBON

Nitrogen and carbon are essential elements commonly found within ecosystems. Both are used in stable isotope studies as tracers for nutrient transference and determinants of plant productivity (Dawson, et al. 2002; Huiskes et al. 2006). N is an essential nutrient, affecting primary productivity due to its limitation in terrestrial systems (Vitousek and Howarth 1991). N can serve as an indicator of primary productivity due to the direct relation between photosynthetic and metabolic rates and soil productivity (Hikosaka and Hirose 2000). Deeply leached soils have been shown to be N limited, since N has a higher rate of leaching, volatilization and denitrification that contribute to increased loss in sandy-island ecosystems (Handley and Raven 1992; Pardo and Nadelhoffer 2010). There is little fractionation of $^{15}N$ during its physical movement across biological membranes, making plant isotope ratios similar to their sources (Dawson et al. 2002). Discrimination is contributed to enzyme-mediated reactions during N assimilation, leaving whole plant $\delta^{15}N$ values relatively similar to their source values (Dawson et al. 2002)**.** On an ecosystem scale, N is lost by migration and denitrification, or transferred back to biotic source pools by ingestion, excretion and decomposition (Pardo and Nadelhoffer 2010).

Carbon isotopes have been primarily used in understanding trophic dynamics and integrated diets for a variety of communities by studying C fractionation during digestion and assimilation (Hilderbrand et al. 1999; Huiskes 2006). Consumer $\delta^{13}C$ values have been closely linked to their prey due to little fractionation (~1‰) within consumer tissues (Schoeninger and DeNiro 1984; Peterson and Fry 1987). C decomposition produces $CO_2$ which escapes or assimilates back into the community with little fractionation within soil (Mizutani and Wada 1988). Effects of C addition have also focused on variability of photosynthetic pathways (Vitousek and Howarth 1991; Dawson et al. 2002). $C_3$ and $C_4$-CAM plant species exhibit a disparity between their $\delta^{13}C$ values due to their differing physiology, having lower (i.e., -30‰ to -20‰) and higher (i.e., -20‰ to -10‰) values, respectively (Peterson and Fry 1987; Farina et al.

2003). $\delta^{13}C$ values have been used as indicator variables for numerous landscape properties such as intrinsic water availability or efficiency (e.g., $^{13}C$ plants for stomatal conductance), ambient $CO_2$ concentration, elevation, and site age (Dawson et al. 2002; Farina et al. 2003; Crait and Ben-David 2007). $^{13}C$ can be a useful tool in examining an organism's dietary dependence on marine and terrestrial environments, or a combination of both. Ramsey and Hobson (1991) found polar bear C isotope values (-17.7‰ $\delta^{13}C$ muscle) to be closely related to values of ringed seals (-18.1‰ $\delta^{13}C$ muscle) and not terrestrial fruits (-26.2‰ to -27.8‰), displaying their primary dependence on marine sustenance. In the construction of an isoscape, $\delta^{13}C$ can be used in conjunction with $\delta^{15}N$ because of its relatively consistent use in determining trophic positioning (Jaeger et al. 2010). Overall, by using $\delta^{13}C$ and $\delta^{15}N$ an understanding of the assimilation of allochthonous nutrients within the biological community is possible if nutrient sources are isotopically distinct.

## 1.3 ISOTOPES WITHIN SOILS AND PLANTS

Sampling soils at various depths can provide a temporal record of nutrient distributions and concentrations, but temporal variability depends on the soil type and the environmental conditions. Deep-vested, organically layered soils with less nutrient leaching provide clear temporal records of nutrient deposition, while sandy-environment systems are plagued with shorter time-frames due to intense nutrient leaching and disturbance. Erskine et al. (1998) found concentrations of soluble N to be four times higher in soils sampled near marine mammal and avian colonies in peak periods of usage; however, this concentration decreased over time indicating the successive degradation of the isotopic signatures. This suggests that plant and soil $\delta^{15}N$ values can differ greatly, depending on the period of sampling (e.g., optimal growth, reproduction, seasonal storing, etc.) and interactions between plant and soil biogeochemistry (Dawson et al. 2002**).** While soil isotope values indicate current nutrient fluxes, plants (i.e., primarily leaf tissue) provide a valuable temporal record of the nutrient content and their interactions with the surrounding abiotic and biotic environment (Erskine et al. 1998; Dawson et al. 2002). Using plants isotopes within environments where the effects of leaching and disturbance are pronounced can provide a broader temporal window in understanding nutrient retention within communities.

Plants display variability in N uptake between both ammonium ($NH_4^+$) and nitrate ($NO_3$). Since the assimilation of inorganic N through atmospheric deposition occurs through both chemical species, inter- and intra-variability among species is attributed to either the availability of the chemical species or physiological preference (Robinson 2001). Legumes (Fabaceae) with mycorrhizal symbiotic interactions provide additional sources of N through $N_2$ fixation (Handley and Raven 1992). These interactions between plants and nitrogen fixers are essential in providing organic forms of N; however, persistent environmental limitations (e.g., leaching, erosion, saltation, etc.) and plant-species competition produce a consistent nutrient deficit in sandy-island ecosystems (Vitousek and Howarth 1991). Terrestrial plant growth, biomass allocation (i.e., shifting between roots to shoots and leaves) and photosynthetic capacity are affected by N concentrations, causing increased competition for the limited nutrient (Tilman 1986; Vitousek and Howarth 1991; Handley and Raven 1992; Atkin 1996). Finally, N transformation within plants occurs during assimilation and amino acid or protein synthesis while N is lost via volatilization from leaves, herbivory, and root efflux (Robinson 2001).

Numerous factors can accentuate isotope variations within plant tissues, including mycorrhizal interactions with plants, depth of root extraction of nutrients, heterogeneity of nutrient concentration within plant tissues, and the form of nutrient uptake (i.e., inorganic or organic) (Handley and Raven 1998). Species display specificity towards isotopic compositions within their tissues due to differing physiology and biochemistry (Lee et al. 2009). Intrinsic water-use efficiency (i.e., ratio of $^{13}C$ assimilation to transpiration) amongst species can be a major cause for isotopic variations within plant tissues and biogeochemistry depending on water availability (Lee et al. 2009). Yet, intraspecific variations are smaller when focus is shifted to examining spatial variability across landscapes where environmental conditions generate greater variability (Lee et al. 2009). Plant and animal tissue $\delta^{13}C$ and $\delta^{15}N$ values provide a time-integrated dietary record of their assimilated diet due to their bio-accumulation within the lifetime of their tissues (Hobson and Clark 1992). Since differing tissues vary in their metabolic rates, tissue-specific isotopic turnover rates change depending on the tissue sampled. By accounting for isotopic turnover rates within plant tissues an accurate assessment of the temporal isotopic variations can be gained by determining specific time periods of sequestering.

## 1.4 IDENTIFYING SOURCES OF NUTRIENTS

Identifying sources of C and N in plants is difficult due to inherent inter- and intra-group differences in uptake, natural history and species specificity (Huiskes et al. 2006). Maguas et al. (1993) found that 90% of carbon isotopic variation within the plant community could be attributed to species specificity and not environmental factors (Galimov 2000). This result could be site dependent, since environmental factors have been shown to be dominant in nutrient-deficient sites (Evans and Ehleringer 1993). Isotopes of sinks ($\delta_{sink}$) track their sources ($\delta_{source}$), but the relationship is not constant if multiple sources with distinct isotopic signatures contribute to the inclusive $\delta_{sink}$ value. Using $\delta^{13}C$ and $\delta^{15}N$ to evaluate C and N inputs from multiple sources to plants is complicated by the processes of mixing and fractionation which degrade clear isotopic signatures (Robinson 2001). Any values obtained are a result of the combined effects of numerous interacting sources and their associated properties (e.g., isotopic fractionations, nutrient gains, nutrient turnover rates, isotopic pool mixing, etc.) would not be accurate in identifying individual sources. This can result in site-specific variation, leaving individual identifying procedures irrelevant unless sources can be isotopically identified and isolated from one another (Crait and Ben-David 2007). However, if isotope values for potential dietary sources are verifiably distinct, then a primary source can be determined by examining plant and soil values (Stapp and Polis 2003). Bokhorst et al. (2007) examined the input of N into three island communities from three independent sources: precipitation, sea spray, and seabird guano. Significant variations were determined between the source's $\delta^{15}N$ values which allowed them to identify individual contributions to N deposition for each individual source (Bokhorst et al. 2007). Natural stable isotope tracers are currently limited in their application of identifying similar source inputs, but identifying verifiably distinct sources is achievable (Robinson 2001).

Marine-derived nitrogen isotope values differ from those originating from terrestrial environments, thereby allowing a basis for determining their source origins (Schoeninger and DeNiro 1984; Peterson and Fry 1987). Heavier isotopic species (i.e., $^{15}N$ and $^{13}C$) are more abundant in oceanic waters than in the atmosphere ($\delta^{15}N$ = 1‰ lower and $\delta^{12}C$ = 7‰ lower), consequently causing marine organisms and their consumers to possess higher isotope values (Ramsey and Hobson 1991; Anderson and Polis 1998; Erskine et al. 1998). Marine sources are

usually higher in $\delta^{15}N$ than terrestrial sources, which are displayed in both the animals feeding in marine food-webs and within plants utilizing marine-derived nutrients (Schoeninger et al. 1983; Hannan et al. 2007). Anderson and Polis (1998) found significantly higher $\delta^{15}N$ values in coastal spiders and scorpions versus inland species due to the greater presence of marine-derived N. Therefore, marine plants ($\delta^{15}N$ = +7.5‰) and animals (i.e. mammals and birds = +14.3‰) encompass higher $\delta^{15}N$ values than their terrestrial counterparts ($\delta^{15}N$ = +0.31 and +5.9‰, respectively) (Wada et al. 1975; Schoeninger et al. 1983). Erskine et al. (1998) examined the contribution of both atmospheric and penguin guano-derived $NH_3$ within plant tissues on sub-Antarctic islands. Plants growing near penguin colonies exhibited closer $\delta^{15}N$ ($\delta^{15}N$ = +11.1‰) to deposited guano (+14.6‰), indicating plant assimilation of penguin guano (Erskine et al. 1998). Increasing distance from active penguin colonies resulted in decreasing plant values (minimum ~-7‰) that had been closely associated with atmospheric penguin-derived $NH_3$ values (-10‰) within the centre of the colony (Erskine et al. 1998).

When sources are isotopically distinct, their influence on the surrounding community can be quantified with the use of stable isotope mixing models (Hopkins and Ferguson 2012). Mixing models act as tools to estimate proportional contributions of isotopically different resources to the mixture of the isotopic composition within the consumer's tissues to gain inferences on their assimilated diet (Phillips 2012). $\delta^{15}N$ and $\delta^{13}C$ mixing models have been used in a broad range of diet reconstruction studies for a variety of consumers including bats (Painter et al. 2009), bears (Ben-David et al. 2004), cetaceans (Lesage et al. 2010), and numerous fossil mammals (Fox-Dobbs et al. 2007). However, the reliability of mixing models diminishes with decreasing isotopic source differentiation and the lack of including major source pools. Identification of major source pools and their isotope values can provide a basis for comparison in understanding the general contributions and assimilations within a chosen system (Robinson 2001).

## 1.5 ASSIMILATION OF MARINE-DERIVED NUTRIENTS FROM BIOGENIC VECTORS

Recent research using stable isotope analysis has shown that animal populations can be important biogenic vectors for nutrient transfer within ecosystems. Nutrient enrichment of terrestrial ecosystems by biogenic vectors has been shown throughout various biological landscapes, both affecting nutrient availability and total quantity of nutrients sequestered in biological tissues (Anderson and Polis 1998; Stapp and Polis 2003; Hannan et al. 2007). Cocks et al. (1997) showed the significance of migratory snow petrels (*Pagodroma nivea*) in delivering nutrients to nutrient-deficient Antarctic ecosystems. Seabird guano is enriched in $^{15}N$, therefore sites with increased seabird activity display higher overall N concentrations (i.e., 26% higher N concentration within colonies and 6% outside) and higher $\delta^{15}N$ within the surrounding soils (Mizutani and Wada 1988; Cocks et al. 1998; Ellis et al. 2006). Total nitrogen concentrations in the Baja California islands were 2.2 mg $g^{-1}$ total N in soils with seabird activity and 0.3 mg $g^{-1}$ total N in soils without (Anderson and Polis 1999). Transference by seabirds primarily occurs via uric acid which rapidly converts to $NH_3$ and $NO_3$ that plants actively assimilate to directly increase their primary productivity. As seabird densities increase the effects of their deposition can compound, promoting the rapid hydrolysis of uric acid that causes an increase in local pH and promotes ammonia ($NH_3$) uptake by plants (Erskine et al. 1998). Wainwright et al. (1998) also found that Alaskan plant $\delta^{15}N$ values were much higher (22‰) within colonies of seabirds than those outside colonies (11‰). Transfer of nutrients inland by nesting birds has been shown to increase the abundance, spatial extension and diversity of plants (Ryan and Watkins 1989; Cocks 1997; Lee et al. 2009).

Biogenic transfer has been shown in a variety of species, including salmon (Reimchen et al. 2002), sea turtles (Hannan et al. 2007), river otters (Crait and Ben-David 2007), seabirds (Lindeboom 1984; Mizutani and Wada 1988; Cocks et al. 1998) and sea lions (Farina et al. 2003). Biogenic nutrient subsidies can be significant in providing essential nutrients; however, their relative importance has not fully been examined. Black-tailed gull rookeries in Japan have displayed annual inputs of N (53 $g/m^2$), potassium (K = 21 $g/m^2$) and phosphorus (P = 45 $g/m^2$), which were all much higher than most intensive agricultural practices (Mizutani 1984). Results

have shown the biogenic transfer of nutrients across ecosystem boundaries to highly affect the structure and resource availability within communities (Ellis et al. 2006). Trophic dynamics can be highly affected by these inputs, by creating a further dependence on marine-food webs by opportunistic species and potentially stabilizing communities (Anderson and Polis 1998). Low to moderate input of allochthonous nutrients can potentially stabilize trophic dynamics by maintaining longer food chains; however higher inputs can lead to instability through intense enrichment (Huxel et al. 2002). Additional research is needed in both quantifying the importance of biogenic vector species to nutrient cycling and in understanding the dependence on marine-derived nutrients by inhabitants of island communities.

## 1.6 CONSIDERATIONS AND LIMITATIONS OF BIOGENIC VECTORS

Cyclical movement of nutrients and organisms are common processes responsible for stabilizing and reinventing ecosystems (Nakano and Murakami 2001). The transporting of nutrients from lesser to higher productive systems depends on both the landscape's permeability and the characteristics of abiotic or biogenic vectors (Huxel et al. 2002; Farina et al. 2003). The characteristics of a biogenic vector can greatly influence nutrient transfer, such as differences in physiology, variations of behaviors and the mobility of the species (Farina et al. 2003). Therefore, both rates and total nutrients transferred are highly affected. An organism's trophic position has been linked to the $^{15}N$ enrichment within their tissues. The higher the trophic level the more $^{15}N$ enriched the tissues and excreta, displaying a characteristic 2–4‰ increase with each trophic level (Erskine et al. 1998; Eggers and Jones 2000). Differences in $\delta^{15}N$ values in two species of sea turtles, loggerhead (10.6‰) and green (6.0‰), were linked to their differing trophic levels (Hannan et al. 2007). This is primarily due to isotopically lighter $^{14}N$ being excreted as excess dietary waste (Adams and Sterner 2000).

Permeability of the habitat can greatly reduce biogenic transfer if the topography limits (i.e., impassible terrain) the mobility of the species (Farina et al. 2003). Farina et al. (2003) found the transfer of nutrients to be spatially restricted depending on the mobility of the biogenic vector species. Influence of the Galapagos sea lion (*Zalphus wollebaecki*) was restricted to shorelines with low elevations (<20 m), indicating how landscape characteristics can affect nutrient transfer

(Farina et al. 2003). This presents an important relation in regards to biotic vectors— with increased mobility there is an increase in the spatial extension of their nutrient transport within terrestrial ecosystems. Shoreline cliffs are relatively impassable for low-mobile species (e.g., turtles, penguins, sea lions, etc.) and minimally passable for highly-mobile species (e.g., gulls, seabirds, etc.), which creates variability in nutrient transfer between vector species (Farina et al. 2003; Lee et al. 2009). Thus, the spatial extension and the localized concentration of nutrients will depend on the physical movement of a species (Cocks et al. 1997).

Reduced-flight or flightless birds, such as cormorants and penguins respectively, exhibit more concentrated and less spatially extended values than their flight-able counterparts (Mizutani and Wada 1988; Erskine et al. 1998; Ellis et al. 2006). The density of the biogenic vector species is directly related to the increased levels of N and higher $\delta^{15}N$ within the surrounding soils plant communities (Reimchan et al. 2003). Preliminary studies on nutrient concentrations within soils surrounding snow petrel colonies determined N, K and P levels to decrease further away from the colony (Ryan and Watkins 1989). The contribution of urea or $NH_3$ by higher trophic consumers is more significant than other material due to its decreased localization and rapid rate of utilization by numerous soil-leaching species (Hilderbrand et al. 1999; Crait and Ben-David 2007). Although this relation suggests that an increase of primary productivity in colony centroids is expected, high levels of N and P concentrations can inhibit growth, change trophic dynamics, and increase plant mortality (Hogg and Morton 1983). Deleterious $NH_3$ levels can inhibit both cation and $NO_3$ uptake which increases anion content within tissues and acidifies the surrounding soils (Ellis et al. 2006). It has also been speculated that areas with high concentrations of nutrients can exhibit intense disturbance by maintaining early successional stages for plant communities, such as grasses (Ellis et al. 2006). Areas highly concentrated with biogenic vector species (e.g., penguins, seabirds, seals, etc.) consistently exhibit less plant richness and density, due to the effects of intense nutrient enrichment (Mizutani and Wada 1988; Cocks 1997; Farina et al. 2003). In island ecosystems, N within urea and excrement rapidly mineralizes to release $NH_3$ gas and is either blown away or leached into the sea (Mizutani et al. 1986; Lee et al. 2009). Since the biophysical characteristics of these forms of input vary (i.e., turnover rates, rates of decomposition, mineralization, etc.), their assimilation into the community depends on species utilization.

## 1.7 TEMPORAL AVAILABILITY

The spatial and temporal distributions of animal-derived nutrients vary across landscapes, depending on the limitations presented by the environments. Temporal scales have not been fully considered in understanding the connections of nutrient transfer between communities (Anderson and Wait 2001). Reimchen et al. (2002) illustrated the importance of anadromous fishes in supplying terrestrial ecosystems with an influx of marine-derived nutrients during their spawning season. Results indicated a direct relation between salmon spawning density in coastal watersheds and $^{15}N$ enrichment across the trophic community (i.e., soil, riparian vegetation, wood, scavenger invertebrates, and large predators). This was due to nutrient rich carcasses being transferred into vegetated areas by higher level consumers, primarily the local bear population (Reimchen et al. 2002). These temporal windows for biogenic nutrient transfer are referred to as "nutrient pulses" due to their limited availability and intense localized concentration (Anderson and Polis 1998; Reimchen et al. 2002). Since pulses provide a small window of opportunity, the responsiveness of the trophic community should peak in these periods (i.e., optimal growth periods) to maximize nutrient allocation.

Reproduction is seasonal in many marine mammals and birds, permitting a specific time period (i.e., pulses) for nutrient capturing and sequestering by plants. Total N in penguin island rookeries in Antarctica peaks during their reproductive season in March (TN >7 mg/ml) and then continually declines (TN <1 mg/ml) due to their assimilation by plants and leaching (Lindeboom 1984). Further nutrient radiation into higher trophic consumers depends on the continuing of these massive seasonal inputs, which then alter consumer behaviors and cycles. Nutrient pulses can be drastically affected by environment perturbations (e.g., dramatic temperature changes, El Nino, precipitation changes, etc.), cascading into the trophic dynamics of the ecosystem (Anderson and Polis 1998). In response to these perturbations, some marine mammals and birds have exhibited decreased rates of reproduction between pulses, suggesting their population dynamics may be governed by this temporal availability of nutrients (Duffy 1990; Limberger 1990).

## 1.8 IMPORTANCE FOR NUTRIENT-DEFICIENT ENVIRONMENTS

The ecological importance of nutrient supplements can be related to the bio-availability of nutrients within ecosystems, suggesting nutrient-deficient systems rely more heavily on subsidies than non-nutrient-deficient systems (Bouchard and Bjorndal 2000). In systems where in situ decomposition of organic matter unequally compensates for high precipitation rates and intense nutrient leaching, availability from decomposition is temporally short. Crait and Ben-David (2007) found increased $\delta^{15}N$ values and a larger %N within grasses in latrine sites used actively by otters, thus fertilizing the local environment above its intrinsic capacity. These subsidies could potentially offset high rates of leaching in sandy-soil environments (Hannan et al. 2007). Sand-dune ecosystems experience intensive leaching and high rates of drainage, causing rapid nutrient loss. A significant source of energy and nutrients from allochthonous sources can provide the resources needed to stabilize community structure as well the system. The role of sea turtles as biogenic vectors is isotopically evident in transporting nutrients from marine foraging grounds into sandy-dune systems on the Florida coast (Bouchard and Bjorndal 2000). Leaf tissues, total soil N concentrations, and foliar N concentrations were positively correlated to turtle nest density, suggesting that biogenic vectors support dune stabilization by enhancing the vegetation (Hannan et al. 2007).

If undisrupted, nutrient enrichment can affect the stability of the ecosystem and the processes that encompass it, such as species composition, vegetation density and trophic hierarchies (Crait and Ben-David 2007). Allochthonous sources of macronutrients have been shown to not only limit plant growth and cover, but also increase plant productivity (Anderson and Polis 1999; Farina et al. 2003). Modeling by Huxel et al. (2002) suggested moderate allochthonous nutrient inputs can stabilize the persistence of communities; however, strong inputs destabilize food web dynamics dramatically by increasing consumer populations above their carrying capacities and decreasing primary production by changing the local soil chemistry. Populations of tenebrionid beetles on nutrient-deficient desert-islands were indirectly related to seabird roosting use and the deposition of marine-derived nutrients. Densities were five times greater on roosting islands and six times more within colonies, rather than outside (Sanchez-Pinero and Polis 2000). Antarctic soils continue to be some of the least-developed and nutrient–

poor soils recorded, yet remain relatively productive due to seabird, seal and invertebrate marine-derived nutrient subsidies (Lindeboom 1984; Bokhorst et al. 2007). Therefore, Huxel et al.'s (2002) hypothesis may be limited in incorporating nutrient-deficient systems that potentially combat intense periods of enrichment.

## 1.9 CASCADING INDIRECT EFFECTS OF NUTRIENT SUBSIDIES

Beyond biogenic vector species, the movement of additional terrestrial consumers (i.e., brown bears, otters, horses, etc.) may indirectly enable further penetration of marine-derived nutrients into the interior by their dispersal (Stapp and Polis 2003; Crait and Ben-David 2007). This may indirectly increase the spatial redistribution of nutrients, providing one of many in situ processes decreasing deleterious concentrations. Frank and Evans (1997) identified the relation of ungulate species in grasslands of Yellowstone National Park to their promotion of N cycling and the decrease in accelerated N loss. Deposition of urine and feces promoted plant $NO_3$ assimilation, while grazing pressures contributed to undisruptive N loss through denitrification, $NH_3$ volatilization and leaching (Frank and Evans 1997). Biogenic vectors could potentially bring about an equilibrium, indirectly affecting community richness and stability by affecting their habitat's structure through in situ processes. Large herbivores contribute to nutrient cycling by: producing more available forms of N to plants and soil microbes (McNaughton et al. 1988), increasing detrital turnover rates through increased spatial movement (Ruess 1987), and reducing C:N ratios in litter and soil (Shariff et al. 1994; Frank and Evans 1997). Landscapes encompassing seasonal ranges for a species could be provided with marine-derived nutrients through the seasonal spatial movements and patterns of large organisms. Distributions of nutrients remain spatially concentrated into localized pockets across the landscape, but it is through the indirect transfer of nutrients by these secondary biogenic vectors that nutrients are spatially redistributed.

Allochthonous nutrient subsidies can change the trophic structure of a community, affecting consumers indirectly through the transfer of nutrients into the local food web. Marine-derived subsidies have been directly linked to both plant and animal productivity and density in island communities; however indirect effects of nutrient enrichment have not been examined

(Pearson and Stewart 1993; Stapp and Polis 2003). Stapp and Polis (2003) speculated that spatial nutrient subsidies from biogenic vectors positively affect population density, potentially providing more nutrients then their terrestrial environments. Polis and Hurd (1996) stated that allochthonous resources in island systems are primarily used by detritivores, but recent research has shown their incorporation into higher trophic levels. By examining the presence of marine-derived nutrients within the California Island's omnivorous-rodent populations, Stapp and Polis (2003) found a clear indication of marine-derived nutrients within rodent tissues in small islands with increased seabird activity. Processes of indirect nutrient allocation from biogenic vectors within consumer species are termed "trophic cascading effects" and have been shown in species of spiders (Anderson and Polis 1998), anadromous fishes (Mathewson et al. 2003), bears (Hilderbrand et al. 1999) and ungulates (Frank and Evans 1997). This indirect nutrient allocation compartmentalizes the trophic community, allowing nutrients and energy to flow through different biological pathways through resource partitioning (Huxel et al. 2002).

Although the small localized distribution made rodents unsuccessful conduits for successional marine-derived nutrient transfer, larger organisms with increased spatial movements could further subsidize the community. Redistribution of salmon-derived $^{15}N$ by defecation (3% of total excreted) and urine excretion (96%) by brown bears was found to account for 15.5–17.8% of the total N in spruce foliage within 500 m radius of a active spawning site (Hilderbrand et al. 1999). In relation to spatial patterning and landscape connectivity, more mobile species contribute to larger energetic fluxes across landscapes (Huxel et al. 2002). These fluxes can influence a biological system's structure by heavily influencing either top-down or bottom-up control (Huxel et al. 2002). This would imply that the indirect effects on food-web dynamics by allochthonous inputs are fundamental in determining the density and distribution of recipients in different trophic levels (Huxel et al. 2002). Consequently, biogenic vector species indirectly exhibit "donor-control" over the local community through their allochthonous marine-input (Sanchez-Pinero and Polis 2000). Local consumer dynamics do not affect the renewal rate of the biogenic vector species, causing biogenic vector species to remain relatively unaffected by the local community. Since there is a much greater dependence on biogenic vectors by the local communities (i.e., donor-control), the inhabitants maximize nutrient acquisition during these intense nutrient pulses. The Sable Island horse population could potentially be a vector for

increased nutrient subsidy and heterogeneity through fecal deposition and wide-spread spatial movement. Understanding the role of horses in redistributing marine-derived nitrogen will further aid in understanding nutrient cycling in island ecosystems and the importance of biogenic vectors on higher level consumers.

## 1.10 RESEARCH OBJECTIVES

In order to determine whether marine-derived nutrients were being transferred onto Sable Island by biogenic vector species, a landscape-scaled spatial understanding of N and C was needed. I believe landscape-scaled isoscapes are important tools in understanding nutrient sources and the cycling of nutrients within a variety of ecosystems. Therefore, my first objective was to develop an isoscape for Sable Island, specifically examining $\delta^{15}N$ and $\delta^{13}C$ in vegetation samples using stable isotope analysis. Since nutrients—marine- or terrestrial-derived—would be less persistent in sandy-soils (e.g., inundation, leaching, saltation, etc.), plant tissue samples were used to understand the distribution of nutrients across the island as well as their permeation into the plant community. I expected higher plant $\delta^{15}N$ and lower $\delta^{13}C$ values within seal and tern colonies, primarily concentrated along the island perimeter and tips of the island where seal accessibility was highest. Overall, I anticipated greater variability in $\delta^{15}N$ than in $\delta^{13}C$ since other studies have shown the addition of marine-derived nutrients to primarily affect local N abundance and cycling.

My second objective was to use stable isotope analysis to determine the contribution of both grey seal and tern populations' direct transfer of marine-derived nutrients to the island's vegetative community. I hypothesized that plant $\delta^{15}N$ and total vegetation cover would increase in areas with high biogenic vector densities, primarily grey seal colonies. I predicted that the highly localized distributions of seabirds would show less permeation of $\delta^{15}N$ into the surrounding community and negatively affect total vegetation cover. Finally, I aimed to determine whether the enrichment from biogenic vectors indirectly permeated into the trophic community by tracing marine-derived $\delta^{15}N$ within the local horse population. I predicted horses that resided within the tips of the island would display higher $\delta^{15}N$ values from consuming forage enriched by biogenic vector species, primarily grey seals.

# 2 METHODS

## 2.1 STUDY SITE

Sable Island is a vegetated, crescent-shaped sandbar (~3400 ha) located 275 km southeast of Halifax, Nova Scotia, extending 49 km in length and 1.3 km at its widest (Figure 2.1). Shoreline physiography is shaped by prevailing winds, exhibiting perimeter-foredunes (9–12 m in elevation, max of 25 m) that are separated by blowouts (Beson 1984; Stalter and Lamont 2006). Topography is highly affected by oceanic and wind forces, both contributing to the structure and functionality of the island. Perimeter topography is characterized by large founding and established dunes (10–25 m elevation) that slope inland. Inland topography consists of scattered rolling dunes (mean elevations of 1–10 m above sea level) with depressions that can serve as freshwater ponds. The inland environment has been historically stable due to the shoreline-barrier of dunes that prevent direct influence from salt waters (Beson 1984). Two dune types exist: primary dunes form from *in situ* continuous sand deposition and vegetated secondary dunes which are formed from the migration of lower relief dunes (Byrne and McCann 1995). Dune-environments are fragile habitats, susceptible to rapid change whether environmentally or anthropogenically induced.

The island represents an arid eco-region comprised entirely of accumulated sand from the Sable Island Bank within the Scotian Shelf. Soils are characterized as regosolic, consisting of unconsolidated sand and lacking any organic layers. Climate is temperate oceanic, primarily influenced by the eastern Atlantic Ocean, with winter (January to February) temperatures ranging from +5 to -5°C and summer (July to August) temperatures peaking at 25°C (Stalter and Lamont 2006). Precipitation and prevailing winds are common with the island averaging 137 cm/year of rain and a north westerly average blow of 20 knots. Thick fog commonly covers the island in the summer months and the ready occurrence of low-pressure systems can cause extreme weather conditions throughout the year.

Sable Island supports a diversity of plant species (~171 species) including grasses, forbs and shrubs. The island encompasses ten differing ecological zones covering ~36% of the island: sandwort communities (*Honckenya peploides*), marram-forb grasslands (*Ammophila breviligulata, Lathyrus maritimus, Achillea occidentalis*, etc.), marram grasslands (*A. breviligulata*), marram-fescue grasslands (*A. breviligulata, Festuca rubra, Anaphalis margaritacea, Fragaria virginiana*, etc.), shrub-heath community (*Empetrum nigrum, Rosa virginiana*, etc.), cranberry-heath community, freshwater pools, freshwater pond edges, deep brackish ponds, and shallow brackish ponds (Catling et al. 1984). The remaining area consists of unconsolidated sand with sparse vegetation. For our purposes, the island can be divided into three major vegetation divisions: 1) sandy beaches, 2) perimeter marram-dominated grasslands, and 3) midline heath-shrub communities (Tissier 2011). Vegetation is primarily concentrated inland, leaving the shores and island edges practically barren except for established halophytic communities (e.g., sandwort, marram, etc.) that can withstand intense tidal interactions. The perimeter of the vegetated zone (1–150 m) consists of marram-forb and beachpea (*Lathyrus maritimus*) communities. The midline (100–350 m) of the island encompasses heath and rose communities as well as freshwater ponds that are the primary source of fresh water on the island. With decreasing sand deposition and increased stability, marram-fescue communities are replaced by health-shrub communities which are situated in the middle of the island (Beson 1984). High elevations (i.e., dune peaks) often support dense marram communities. A peak growth period for most plants occurs from late June to the middle of August (Tissier 2011). American beachgrass, or marram grass, is the dominant perennial species on the island primarily growing along the coasts but extending into the island center. Native to eastern North American, marram is a halophytic xerophyte species, thriving in various disturbance regimes within sandy-ecosystems and involved in dune stabilization (Tissier 2011; USDA 2013). Plants spread rapidly through the subsurface by burial-tolerant rhizomes, while stem height can reach two to three feet with multiple stems originating from one clump (USDA 2013). The blooming period begins in early June leading to the formation of a spike-like seed head in late July or early August.

Frequent settlement attempts and shipwrecks have periodically changed the landscape, especially since the establishment of the life-saving station in 1801 (Stalter and Lamont 2006). Agricultural development, planting projects and farming have influenced Sable Island's

community, causing the introduction of non-native species. Since 1959, when the federal government closed the life saving station, the island's human presence has been small with little effect on the local environment. The island remains relatively isolated from human contact except for the continual presence of ~5 staff operating the weather station and support facilities currently funded by Environment Canada's Atmospheric Environment Branch (AEB), as well as the seasonal presence of researchers from the Department of Fisheries and Oceans (DFO) conducting studies on pinnipeds. Regulation for Sable Island is currently under the legislative authority of the Government of Canada and is being managed according to the National Parks Act. Canadian Wildlife Service has also established the island as a migratory bird sanctuary (Beson 1998). Sable Island is currently in transition between Environment Canada protection and gaining National Park Status from the Canadian federal government.

## 2.2 INHABITANTS AND BREEDING COLONIES

Sable Island supports numerous species of invertebrates, 320 visiting species of birds, and a self-sustained population of wild (feral) horses. Sable Island is also a breeding location for terns (common [*Sterna hirundo*], Arctic [*Sterna paradisaea*], and endangered Roseate [*Sterna dougallii*]) and the largest breeding populations of grey seals (*Halichoerus grypus*, >300,000 individuals). Both tern and grey seals occupy the island seasonally as breeding residents, whereas the population of horses are permanent residents.

Grey seals are continental shelf-marine mammals with adults weighing 150–350 kg and ranging in length from 1.5–2.5 m. They feed on a variety of prey species including groundfish and sand lance (*Ammodytes* spp.) in the surrounding waters of Sable Island throughout the year, but primarily spend their time on the beaches and inland areas during the breeding season (Bowen et al. 1992; Beck et al. 2006; Breed et al. 2006). Terrestrial movement is restricted only by steep elevations on the island (Farina et al. 2003). Trillmich (1979) presented the preference of Galapagos sea lions for flat sandy beaches where access to calm water and tide pools was ample. However, with no terrestrial predators, grey seals use much of the island during their breeding season (early December to early February) (Bowen et al. 2003). Although the largest numbers and most extensive use of the island occurs during the winter breeding season,

thousands of grey seals haul out on the beaches periodically throughout the remainder of the year. Grey seals are important in both coastal and shoreline habitats, potentially establishing nutrient gateways due to their large and increasing populations. Transfer of nutrients occurs primarily through either defecation or decay of carcasses from dead seals. Carcasses occur mainly on the beaches but some also occur in vegetated areas of the island (W.D. Bowen, Bedford Institute of Oceanography, pers.comm.).

Sable Island is a roosting and nesting site for tern and gull species, encompassing 2400—3960 and ~3300—4200 breeding pairs, respectively (Beson 1984; Toms et al. 2006). Tern numbers have significantly dropped since the early 1900s (estimated to be over 100,000 individuals), but have remained stable since the 1970s (Horn and Taylor 2000). In 2006, there were thirteen colonies distributed across the island (Toms et al. 2006). This included two primary colonies: one near the main station (Main station colony) and the other (East light colony) at DFO's East Light house (Toms et al. 2006). It is speculated that the decrease in total colonies on Sable from 19 in 2000 to 13 in 2006 has contributed to an increase in the size of the two primary colonies, for reasons not determined (Horn and Taylor 2000; Toms et al. 2006). Average clutch size for terns is ~2.41 (±0.65 standard deviation) across the island but their recent success has been contributed to the two major colonies (Toms et al. 2006). Transfer of nutrients by seabirds occurs via guano, eggs (Siegfried et al. 1978), feathers (Williams and Berruti 1978) and by decomposition of bodies (Williams et al. 1978). These additions may enhance plant productivity and sustain detritus scavenger populations (Cocks et al. 1998; Sanchez-Pinero and Polis 2000). Studies indicate gull and seabird $\delta^{15}N$ values to be roughly 10‰ (Mizutani and Wada 1988; Ellis et al. 2006), but they display variability in soils and plants due to fractionation. The primary form of excreta (i.e., guano) deposited by seabirds is uric acid (80% of total excreta), which is rapidly converted by micro-organisms into $NH_3$ and sequestered by plants and microbes (Lindebloom 1984).

The feral horse population has existed on the island since the mid 18$^{th}$ century and has remained relatively free from human influence (Freedman et al. 2011). Population structure is characterized by persistent, non-territorial bands residing in three distinct groupings (i.e., west, central and east) across the island (Contasti et al. 2012). These three divisions are defined by

available water sources and food resources, with local density being highest in the west where there is greater sandwort and beach pea (Tissier 2011; Contasti et al. 2012). Bands consist of mares (on average 5-6) and their offspring with a dominant stallion that protects them from bachelor groups. Immigration is highest in the middle of the island, stemming from higher emigration rates from the tips (Contasti et al. 2012). The horse population is predominantly affected by local weather with the highest mortality rates observed during severe winters (Welsh 1975). Island-wide censuses by APEL (University of Saskatchewan) from 2008 to 2012 have identified an increase in the population from 375 to 484 individuals (Contasti et al. 2012); however, historical records indicate lower horse densities through their estimated 11 generations with sizes fluctuating between 150 to 400 individuals (Welsh 1975; Lucas et al. 2009). Currently, the horse population is treated as a naturalized species by the Parks Canada Agency.

How the seal, seabird and horse populations affect local nutrient concentrations is important in developing future conservation and resource management strategies for Sable Island and the surrounding Atlantic marine environment. Stakeholders have debated the importance of the horse population against the island's stability, arguing that their impact could gradually destabilize this fragile ecosystem (Beson 1984). Behaviours such as trampling, digging for water, and feeding have been debated to have long-term deleterious effects on ecosystems stability (Proulx and Mazumder 1998; Asner et al. 2004). In the Atlantic, the population of grey seals on Sable Island has been increasing at an annual rate of 12.8% since the 1960s (Bowen et al. 2003; Trzcinski et al. 2006). This increase in seal populations has coincided with the successive increase of the wild horse population, from ~150 to 484 individuals currently, suggesting a positive interaction between these two species (Welsh 1975; Contasti et al. 2012).

## 2.3 SAMPLING METHODS

### *2.3.1 ISOSCAPE SAMPLING*

I collected samples in July and August 2011, which coincided with the peak growing season of the local plant community (Tissier 2011). A set of random plots ($n$ = 480) were generated to determine spatial variability of nutrient concentrations across the island by use of the "generate

random points" tool within Hawths Analysis Tools for ArcGIS 9.3 (Beyer 2004; Figure 2.1). GIS and vegetation maps were provided by David Colville at the Nova Scotia Community College's Applied Geomatics Research Group at the Lawrencetown Centre of Geographic Sciences. I restricted plots to the vegetated portion of the island due to both the greater presence of biogenic vectors and the greater potential for the retention of allochthonous nutrients within the roots of vegetation communities. I located plot sites by using a handheld Global Positioning System (GPS) to within ±5 m from the original coordinates selected by GIS.

I staked the centre at each plot and placed a 1 $m^2$ quadrat within the foliage for vegetation analysis (Figure 2.2). I determined species-specific plant cover (%) and browse (presence/absence) for six of the most common plants: yarrow (*Achillea millefolium*), American marram grass (*Ammophila breviligulata*), red fescue (*Festuca rubra*), beach pea (*Lathyrus maritimus*), smooth-meadow grass (*Poa pratensis*) and the common wild rose (*Rosa virginiana*) (Tissier 2011). I also included sandwort (*Honkenya peploides*) due to its spatial extension into the island tips where there is generally no other plant species. I collected marram foliar clippings from each site if present. Although foliar clippings were collected for each of the seven species previously mentioned, marram was by far the most common species on the island, occurring in 99% of all plots. If more than one marram plant was present the individual closest to the centre of the plot was selected. Plant samples were cleaned to remove excess debris, placed in paper storage envelopes and frozen (-18°C) to prevent desiccation prior to isotope analysis.

I assessed a variety of spatially-explicit environmental variables at each plot (Tables 2.1, 2.2). These variables have proven to be useful determinates of isotopic distributions in other studies. I determined the local topography using a digital altimeter (precision ±1 m) to account for the slope and used a GPS to estimate elevation (precision ±5 m). Vegetation associations (i.e., communities) consisted of six types: marram grassland, marram-forb (i.e., marram and beach pea), marram fescue, shrub-heath, cranberry-heath, and sandwort. Plot position types consisted of eight categories: crest, shoulder, sideslope, toe-slope, valley bottom, depression, blowout and inter-dune slack. Plot stability was visually assessed and given a value ranging from one to five with higher values representing greater stability (i.e., less erosion). I determined the organic depth with use of a cylindrical soil core (25 cm depth × 10 cm diameter). I noted

instances where plots were located near potential vector species (i.e., within tern colony limits and within 1 m of a horse trail), and if plots were positioned near carrion or contained feces of potential vectors.

I collected a variety of spatially-explicit environmental variables through GIS analysis of plots and their relations to potential vector colonies. I geocoded both seal/tern colony densities and distributions by use of ArcMap v.10 (ESRI 2011). Local distributions of seals were surveyed by the Bedford Institute of Oceanography (BIO) using an aerial photograph (orthorectified digital air photo composites) taken during the winter breeding-season of 2009 and plotting individual pups onto a GIS map. The survey examined annual total population size, local densities and spatial distributions for the entire island during the winter breeding season.

I determined the distributions and densities of tern colonies through field identification and GPS mapping. The spatial limits of tern colonies were geocoded using a GPS by circumnavigating the outer extent of the colony and recording their coordinates. Terns intensely defend their colony during nesting periods (June–July), often extending their defense range during this period (Horn and Taylor 2000). Therefore, I determined colony limits by finding their maximal range for each of the cardinal directions. I defined the maximal range as the point at which no anti-predator responses (i.e., increased diving or hovering) were shown. If tern or gull nest sites were abandoned then I mapped the perimeters and added an additional 10 m buffer to account for measurement error. I incorporated 20 m impact buffers around each colony to account for the potential impact on the surrounding community (Toms et al. 2006). In determining the densities of small colonies (1–30 breeding pairs), flush counts are an acceptable practice, by which the total number of individuals in the air are counted proceeding disturbance. A precise measurement of tern densities was not needed, but rather an estimate was used to determine local concentration. For the two major colonies (East light and Main station colonies), I used results from 2006–2010 censuses performed by Canadian Wildlife Service and Environment Canada (Sable Island Preservation Trust 2010).

I determined plot-specific seal densities by establishing 25 m buffers around each plot and counting the number of individual pups situated within. Distances to colonies were determined

by measuring the distance from each plot to the nearest seal or tern colony centroid. Female grey seals and their pups are relatively sedentary while nursing, often remaining in scattered clusters within the vegetation of Sable Island (Boness et al. 1995; McCulloch and Boness 2000). I determined inclusion limits for seal clusters (i.e., colonies) using the "point distance" tool in ArcMap v.10 (ESRI 2011) to determine the average distance (15 m) between seal pups. From this distance, I defined seal colonies as consisting of ≥5 pups within 15 m from colony centroids. I selected a minimum density of 10 individuals (one female for every pup) to incorporate smaller clusters of grey seals within the middle of the island. If more than one colony was positioned within 100 m of the plot then a level of permanence was determined for each colony by surveying the number of pups within the surrounding colonies (Farina et al. 2003). Colonies that produce more pups will be less transient then colonies that do not, potentially increasing the temporal transfer of nutrients; therefore, colonies with greater pup densities were selected. I included both variables within the isoscape, as well as in separate analyses to determine the direct effects of seal colonies on the plant isotope values. Both distance to shore and the vegetation edge were determined by measuring the shortest distances from the plots to the edges of the corresponding layers.

### *2.3.2 EFFECTS OF BIOGENIC VECTORS ON VEGETATION*

I employed a random-stratified sampling design to examine the local effects of the largest tern colonies on the plant communities. A set of random plots was generated for each colony (i.e., Main station $n = 17$, East light $n = 3$) and an alternative site ($n = 20$) possessing roughly the same community type (i.e., shrub-heath), topography (i.e., elevation, slope, etc.) horse presence (i.e., limited signs of browse or trails) and seal influence (i.e., similar distance to seal colony and densities). I mapped the spatial limits of the alternate site using GIS and then assessed its characteristics using these variables to ensure its similarity. I collected marram foliage samples and recorded plot characteristics at each plot using previous methods. To assess the overall availability of total nitrogen, $NO_3$ and $NH_4$ in the surrounding soils, I used plant-root-simulator $(PRS)^{TM}$-probes (Western Ag Innovations Inc., Saskatoon, SK, Canada). The probes act as a natural ion exchanging resin membrane, similar to plant roots, by continually sequestering biologically-essential ionic species. $PRS^{TM}$-probes provide a direct understanding of the ion flux

within soils and the nutrient supply rates for plants. Probes were inserted in randomly selected plots within the tern colony ($n$ = 6) and non-colony ($n$ = 6) sites from July 20 to August 20 to account for a time period of one month during the peak roosting season. Probes were then washed and placed in containers for transportation to Western Ag. Innovations for analysis. Tern colony centroids were calculated using the "calculate geometry" tool within ArcMap v.10 (ESRI 2011) and distances from each plot were measured.

Plots used for the development of the isoscape were also used in determining the effect of potential vector species on the plant isotope values across the island. I defined each plot as either being ($n$ = 157) or not being ($n$ = 125) influenced by a vector species and further defined by the vector species of influence, using the following criteria: 1) plots designated as having the influence of horses ($n_{horse}$ = 66) either possessed horse feces within the plot or were situated within 5 m of a horse trail and exhibited a large amount of browsed vegetation; 2) plots designated as having the influence of terns or gulls ($n_{bird}$ = 27) either contained guano within the plot or resided within either the colony itself or the surrounding impact buffer; and 3) plots selected as having the influence of seals ($n_{seal}$ = 64) were either within 10 m of a seal carcass or were located near seal colonies. The concentration of nutrients from vector species to the surrounding community is directly related to both the density and distance from the vector species. My preliminary results showed $^{15}$N enrichment of marram grass surrounding a seal carcass to extend beyond 10 m ($\Delta\delta^{15}N = 4.4‰$); however, changes in the local topography (i.e., altitude, dune presence, etc.) can limit further permeation of nutrients into the surrounding communities (Cocks et al. 1998; Lee et al. 2009). To account for this, I defined plots as having the influence of seals if they occurred within 25 m of a seal colony. Upon further assessment some plots were situated below the distance to colony and density requirements, but possessed seals within 10 m of the plot. Cocks et al. (1998) found the greater $^{15}$N enrichment of soil and lichen species to persist 10 m downslope from snow petrel (*Pagodroma nivea*) colonies. To account for this highly localized effect, I established a secondary 10 m buffer around each plot. If the plot failed the previous requirements but possessed a seal pup within the 10 m buffer then it was defined as having the influence of seals. I assigned influence as a mutually exclusive category (i.e., only one species could influence one plot) to simplify analyses, selecting the category for multi-influenced plots ($n$ = 14) firstly on the presence of a species' feces. Although

this is an oversimplification of the system on Sable Island, I stratified data as such to simplify the analysis. Final definitions on the influence of each plot were summarized as a categorical, mutually exclusive variable and used for further analysis.

To determine the differences between the isotopic signatures of each potential vector species, I collected a variety of biological samples for isotopic analysis. Skin samples were collected from the rear flipper of adult beached seals ($n$ = 14), stored in sealed plastic bags and frozen until analysis (Tucker et al. 2007). Adult tern ($n$ = 20) and gull ($n$ = 20) feathers (i.e. coverts) were collected from carrion and stored in sealed plastic bags until analysis. Horse hair (e.g., mane and tail samples ($n$ = 60) were collected by opportunistic pulling of hair, following the University of Saskatchewan Animal Care Protocol (no. 20090032). Hair samples were also collected via scratching posts (mainly washed-up logs and some previously established fence posts) using one-sided velcro tape. I identified the horses that were sampled using their horse ID and location centroid obtained from the population and GIS horse database constructed by the Animal Population Ecology Lab (APEL). I determined distances from the tips of the island for each horse using measuring tools with the ArcMap v.10 software.

### *2.3.3 ISOTOPE ANALYSIS*

I randomly selected 282 marram foliage samples from the total number of samples collected ($n$ = 480) for isotopic analysis. I rinsed samples in distilled water to remove debris, oven dried at 65°C for 24 hrs and powdered. Since marram possessed relatively low amounts of N and high C, I used 12 mg and 1mg aliquots (±0.05 mg) for $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis, respectively. I cleaned horse hair and feather samples of surface oils using a solvent mixture of a 2:1 ratio of chloroform to methanol and dried under a fume hood for 24 hrs. Average hair growth for mammals is 1 cm per month (Bol and Pflieger 2002); therefore, I cut 4 cm subsamples from the follicle upwards to account for nutrient integration within tissues since March 2011. The tips of each feather were cut up to a maximum of 5 cm. Seal skin samples were freeze-dried and repeatedly rinsed in 2:1 chloroform to methanol solvent for lipid removal (Ramsey and Hobson 1991). All samples were then cut, pulverized and subsampled to 1 mg aliquots. I homogenized and weighed samples into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis.

Isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) were assessed by continuous flow stable isotope–ratio mass spectrometry (CF-IRMS) by the Stable Isotope Hydrology and Ecology Research Laboratory at Environment Canada in Saskatoon, Saskatchewan. The subsamples were combusted in a Robo-Prep elemental analyzer and the resulting $CO_2$ and $N_2$ within the samples were analyzed by the Europa 20:20 continuous-flow isotope ratio mass spectrometer. Results were reported in per-mil units (‰) and given the delta notation (δ). Samples were compared to standards for both $\delta^{13}C$ and $\delta^{15}N$, in the form of Vienna PeeDee Belemnite (VPDB) limestone and atmospheric $N_2$ (air), respectively. Isotope values are expressed in a ratio of heavy to light isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) using the following equation:

$$\delta X\text{-}std = \left(\frac{Rsam}{Rstd} - 1\right) 1000(‰) \quad \text{.....(2.1)}$$

where $\delta Xstd$ (either $\delta^{13}C$ or $\delta^{15}N$) is the isotopic ratio in delta units (δ) and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in either the sample or standard.

## 2.4 STATISTICAL ANALYSES

### *2.4.1 ISOSCAPE DEVELOPMENT*

#### *2.4.1.1 INTRODUCTORY ANALYSES AND KRIGING*

I constructed the GIS-based isoscape surface interpolation model, from point sourced geographic assignments of the marram foliage values, using the Geostatistical Analyst extension and geoprocessing tools within ArcGIS v. 10 as well as R v. 2.6.2 (R Development Core Team 2008). Kriging is a useful geostatistical interpolation tool that uses the autocorrelation and spatial relationships within a dataset to develop a probability surface for a variable of interest. By use of grids, isotope values can be spatially extended to extrapolate values for unmeasured, but related, grids by autocorrelation. Relations can then be visually expressed using contour divisions. Plotting the series of semivariance values ($y$) against the dependent lag distances ($h$ = distance between points as a function of autocorrelation) will display differing trends between both groupings if they exist. A simple expression if this relation can be stated as:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(i) - z(i+h)],^2 \quad (2.2)$$

where $N(h)$ is the number of sample pairs that the lag distance ($h$) separates. $Z(i)$ is a known value at location $i$ while $Z(i + h)$ is a known value at location $i + h$ (Wang et al. 2010).

To determine if $\delta^{15}N$ and $\delta^{13}C$ followed the kriging assumptions, I mapped and examined both variables using a variety of exploration tools and analyses. Voronoi maps (i.e., simple cubic lattice) showed a high degree of variability within the spatial distribution of $\delta^{15}N$ with higher values concentrated at both the west and east tips of the island as well as the northern shoreline. The map for $\delta^{13}C$ displayed a lessened variability with no obvious trends within the distribution. I used a 3D-trend analysis to assess the presence of preferential sampling or spatially-explicit trends within the data. Analysis showed no clustering of samples in the distribution of plots; however, an increasing trend was found in higher $\delta^{15}N$ values at the tips of the island. In kriging techniques the variance within the selected variable is divided into that explained by deterministic trends and that attributed to random spatial autocorrelation. These deterministic trends are sequentially removed to allow for the residuals to be modeled separately and then added during the final interpolation procedure. To account for this trend, a second order polynomial was fit to the $\delta^{15}N$ with an exponential kernel function. I optimized the trend removal to allow the removal parameters to be a function of the final cross validation statistics. I examined spatial autocorrelation with use of a semivariogram plot which examined the variance between samples (i.e., squared difference) that are separated by different distances. Larger distances between plots should display higher variances, which is the basis behind spatial interpolation of unknown sites. Although $\delta^{15}N$ displayed functional spatial autocorrelation, $\delta^{13}C$ showed a uniform spatial structure suggesting homogeneous variance across all distances between plots.

*2.4.1.2 CO-KRIGING APPLICATIONS*

To improve the models validity and optimize the kriging interpolation, I considered numerous environmental variables for co-kriging using a variety of statistical methods (Tables 2.1, 2.2). Co-kriging increases the potential for more accurate interpolations by incorporating the relations between secondary variables that explain significant amounts of variance within the variable of

interest. Incorporating these additional variables provides a further understanding of the underlying environmental gradients that affect the distributions of isotopic species across the island.

Prior to modeling, I explored the data using steps defined by Zuur et al. (2009) to ensure all variables met statistical assumptions. I applied appropriate transformations when needed to linearize relationships with the dependent variables (Table 2.1). Levene's tests and conditional boxplots were used to assess homogeneity of variances across the multiple levels. Collinearity between independent variables was assessed using pairwise scatterplots and correlation coefficients (elimination correlation $\geq 0.4$) (Graham 2003; Zuur et al. 2009). I assessed the removal of a correlated variable by individual based regression analysis and review of the literature to eliminate multi-collinearity and model over-parameterization.

I determined variable importance by multiple simple linear regressions and significant variables were incorporated in subsequent models (Tables 2.1, 2.2). I assessed their final inclusion within the model using multiple scatterplots and conditional boxplots to examine the relations between response and predictor variables. Both plot position and community type were removed from the analysis due to either failing the assumption of homogeneity of variance between factor levels or having the variance being explained within a covariate (i.e., community type gradient explained by distance to shore with correlation coefficient = –0.6).

I used general linear models (GLM) to model $\delta^{15}N$ and $\delta^{13}C$ individually using important spatially dependent environmental covariates and their interactions. I performed model selection on the maximal regression models using stepwise Akaike Information Criterion (AIC) until a final parsimonious model was fit. I assessed the overall fit of the model with Q-Q plots of the residuals and by regressing the predicted against observed values. None of the variables explained a significant amount of variance within $\delta^{13}C$. Due to the lack of spatial autocorrelation, lack of predictability from potential covariates and reduced variability within $\delta^{13}C$, an interpolation surface was not used for further analysis (Figure 2.4). I included variables found to explain a significant ($P < 0.05$) amount of variation within $\delta^{15}N$ in the development of the isoscape. I added additional covariate information within the remaining plots that were not used

in the isotope analysis ($n$ = 198) to the dataset. The additional plots increased the spatial information for calibrating the final model.

*2.4.1.3 ADJUSTING KRIGING PARAMETERS AND CROSS-VALIDATIONS*

I used universal kriging in developing the isoscape due to its incorporation of second-order polynomial trends and its flexibility in measuring differing means across large distances. I also constructed an ordinary kriged surface with second-order polynomial trend removal to compare against universal kriging; however, by comparing the cross-validation results universal kriging proved the better option in explaining the greater spatial variability in $\delta^{15}N$. I optimized the resulting semivariogram, from which the interpolated surface was derived from, by performing a series of cross-validations. Optimization is an iterative process that fits numerous semivariogram parameters (e.g., nugget, sill, etc.) by weighted least-squares to determine optimal parameter values. This method provided the most accurate predictions of semivariogram parameters in the resulting model. To account for the multi-directional trends (i.e., anisotropy) at the tips of the island, I fit individual plots on the semivariogram using a variety of directionally-dependent models (Hobson et al., 2009). This strategy optimizes plot-corresponding kriging weights based on their locations on the island and the related trends. I set the lag size to the average nearest neighbor (i.e., 164.2 m) determined by the "average nearest neighbor" function within the spatial statistics tools for ArcMap v.10 (ESRI 2010).

I employed a four-sectored, standard-neighborhood type with defined maximum ($n$ = 5) and minimum ($n$ = 2) neighbors. The search strategy defines how many data points are incorporated in assigning values to unsampled locations using the "moving window" search neighborhood. I used the post-hoc cross-validation results from the model to quantify the uncertainty of predictions and model performance. Each data point was progressively removed in order to predict the selected value from the model and develop prediction errors for each point. Out of the numerous models constructed, I selected a final model that minimized the cross-validated Root Mean Square (RMS) error and possessed the most accurate regression results comparing observed versus predicted $\delta^{15}N$ (Hobson et al. 2009). Once the final model was created, I determined the relation between the density of seals and marram isotope values by spatially

joining the two layers and creating a count field to determine the number of seals within each isotopic range.

### *2.4.2 INFLUENCE OF BIOGENIC VECTOR SPECIES*

All statistical tests were performed at an assumed significance level of $P < 0.05$. I assessed normality within continuous variables using Q-Q plots and Kolmogorov-Smirnoff tests while Levene's test and conditional boxplots were used to determine homogeneity of variances within categorical variables. I removed outliers in the data based on boxplots and the researcher's judgment.

I compared plant $\delta^{13}C$ and $\delta^{15}N$ between tern colonial ($n$ = 20) and non-colonial sites ($n$ = 20) using individual analysis of variances (ANOVAs). I assessed total vegetation cover and species richness within plots using the same methods. Since the soil nutrient values from PRS™-probe analysis failed the homogeneity of variance assumptions, I employed Mann Whitney U tests to compare values between the two sites. I logarithmically transformed the distances from each plot to colony centroids to meet statistical assumptions and regressed against $\delta^{13}C$ and $\delta^{15}N$ using individual GLMs to determine the spatial localization of nutrient inputs.

To determine if vector species were introducing greater $^{15}N$ and $^{13}C$ into the surrounding vegetation within colonial areas, individual ANOVAs were used to compare isotope values of marram grass for colonial ($n$ = 157) versus non-colonial ($n$ = 125) influenced samples. I then employed an ANOVA with post-hoc contrast coding tests to determine if there were differences in marram $\delta^{15}N$ values between sites influenced by individual biogenic vector species. Vector-influenced plots consisted of those with non-colonial influence ($n$ = 125), horse influence ($n$ = 66), tern influence ($n$ = 27) and seal influence ($n$ = 64). I used these same methods to determine differences in the total vegetation cover and species richness for each of the influence types. However, since these variables were strongly affected by the local community type, only marram-grassland plots ($n$ = 217) were used in the analysis. I divided these plots into non-colonial ($n$ = 102), horse influenced ($n$ = 51), tern influenced ($n$ = 18) and seal influenced ($n$ = 46) plots. The relation between plot-specific seal density and the total vegetation cover within

plots was determined using a GLM. Since the data did not meet the assumptions of an ANOVA, I tested the effect of guano/feces presence on foliage $\delta^{13}C$ and $\delta^{15}N$ using a Kruskal-Wallis test with comparisons (Zurr 1999). Individual linear regression analyses were also used to compare $\delta^{13}C$ and $\delta^{15}N$ with seal densities and distances from colonies.

Individual ANOVAs with post-hoc contrast coding tests were employed to determine if horse $\delta^{13}C$ and $\delta^{15}N$ values differed between the east tip ($n$ = 15), west tip ($n$ = 15) and middle ($n$ = 30) of the island**.** I defined the transition from the tips to the middle by visually examining the spatial distribution of seal pups and area-specific P/A ratios (Figure 2.3). Results from Farina et al. (2003) showed higher P/A ratios to significantly increase island occupation and the potential for nutrient transfer by sea lions. Therefore, the divisions I used promoted higher P/A ratios (P/A >5) and greater seal permeability (i.e., defined by greater densities) within the tips of the island. These divisions allowed me to determine the influence of seal-derived $^{15}N$ on horse isotope values by comparing areas with different measures of seal influence. The divisions roughly, though not exactly, corresponded with the three divisions identified by Contasti et al. (2012) to divide the island according to the clustering of water and vegetation resources for horses as well as female movements (2008–2012). I regressed horse isotope values against their corresponding distance from either the east or west tips using a GLM. Additional ANOVAs with the same divisions were employed on the marram $\delta^{13}C$ and $\delta^{15}N$ values as well as total vegetation cover to determine whether any differences in horse values were attributed to their forage. Since the sample sizes between the middle and the tips of the island were unbalanced, a random selection of samples ($n$ = 30) were taken from each division. I also calculated local seal and horse densities as well as perimeter-area ratios for each division.

To determine whether horses at the tips of the island sequestered colonial-influenced marram within their tissues, stable isotope mixing models were implemented using IsoError (Phillips and Gregg 2001). IsoError determines contribution estimates for individual sources with confidence intervals that reflect the amalgamation of sources within the consumer (Phillips and Gregg 2001; Phillips et al. 2005). Separate models were constructed for horses foraging in the tips ($n$ = 30) and the middle ($n$ = 30) of the island. Two sources, vector-influenced (i.e., seal and tern influenced plots = 6.8 ± 4.1‰) and terrestrial-influenced (i.e., horses and non-influenced

plots = 1.8 ± 2.8‰) marram grass, were partitioned with horse isotope values. Due to the lack of variability in source $\delta^{13}C$ values (biogenic influenced plants = -27.4 ± 2.0‰, non-influenced plants = -27.1 ± 1.5‰), a simple linear mixing model was used to examine the incorporation of source $\delta^{15}N$ within the horse population. A diet-hair $\delta^{15}N$ tissue-fractionation factor of 3.25 ± 0.4‰ was subtracted from the average horse $\delta^{15}N$ to account for trophic enrichment (Sponheimer et al. 2003; Mannel et al. 2007).

**Table 2.1**: Transformation type, summary values (i.e., standard deviation, mean ± standard error), and simple regression statistics for plot-specific ($n$ = 282) environmental covariates against marram $\delta^{15}N$ values (Sable Island, Nova Scotia, 2010). Transformations were applied to improve linearity between covariates. Significant variables were included within the general linear model which was further simplified.

| **Covariate** | **Transform** | **SD** | $\mu$ | $F_{1,280}$ | $r^2$ | $P$ |
|---|---|---|---|---|---|---|
| % Beach Pea Cover | log | 18.9 | 8.5 ± 1.5 | 0.2 | 0.01 | 0.341 |
| % Marram Cover | log | 37.3 | 55.7 ± 2.2 | 5.8 | 0.02 | 0.02 |
| Distance from Seal/Tern Colony (m) | log | 62.8 | 72.4 ± 3.7 | 157.4 | 0.36 | < 0.001 |
| Distance from Shoreline (m) | log | 159.4 | 265.5 ± 9.5 | 7.6 | 0.03 | 0.006 |
| Distance from Vegetation Edge (m) | log | 117.9 | 138.4 ± 7.0 | 9.6 | 0.03 | 0.002 |
| Elevation (m) | log | 4.7 | 11.6 ± 0.3 | 0.3 | 0.01 | 0.581 |
| Height of Vegetation (cm) | none | 12..4 | 46.2 ± 0.7 | 1.6 | 0.01 | 0.207 |
| Seal Density (25 $m^2$) | log | 7.8 | 4.7 ± 0.5 | 85.9 | 0.23 | < 0.001 |
| Slope (°) | log | 7.5 | 5.7 ± 0.4 | 0.1 | 0.01 | 0.805 |
| Species Richness (# species) | square-root | 2 | 3.2 ± 0.1 | 8.9 | 0.03 | 0.003 |
| Total % Vegetation Cover | none | 27.9 | 60 ± 1.6 | 0.3 | 0.01 | 0.606 |

*Note*: Values with $P < 0.05$ were included within maximal model.

**Table 2.2**: Descriptions, number of levels and simple regression statistics for plot-specific environmental categorical variables against marram $\delta^{15}N$ values (Sable Island, Nova Scotia, 2010). Significant variables were included within the model to be further simplified.

| Categorical Variable | Description | Levels | *F* | *P* | $r^2$ |
|---|---|---|---|---|---|
| Browse (p/a) | Presence of horse browse | 2 | $F_{1,280} = 4.7$ | 0.031 | 0.02 |
| Guano / Feces (p/a) | Presence of guano/feces | 2 | $F_{1,280} = 1.8$ | 0.174 | 0.01 |
| Plot Position | Description of local topography | 8 | $F_{7,274} = 2.7$ | 0.003 | 0.09 |
| Plot Stability | Stability of soil within plot | 5 | $F_{4,277} = 1.2$ | 0.322 | 0.02 |
| Vegetation Community | Vegetation community type | 6 | $F_{5,276} = 2.9$ | 0.003 | 0.08 |

*Note*: Values with $P < 0.05$ were included within maximal model.


**Figure 2.1**: Map of Sable Island, Nova Scotia, displaying the vegetated surface (i.e., green), sand surface (i.e., beige) and plot locations ($n$ = 480).

**Figure 2.2**: Photo of a typical 1 $m^2$ plot at an average grassland community site. Species-specific and environmental plot attributes were recorded within the limits of the plot boundary. Photo by Kenton Lysak, 2010.

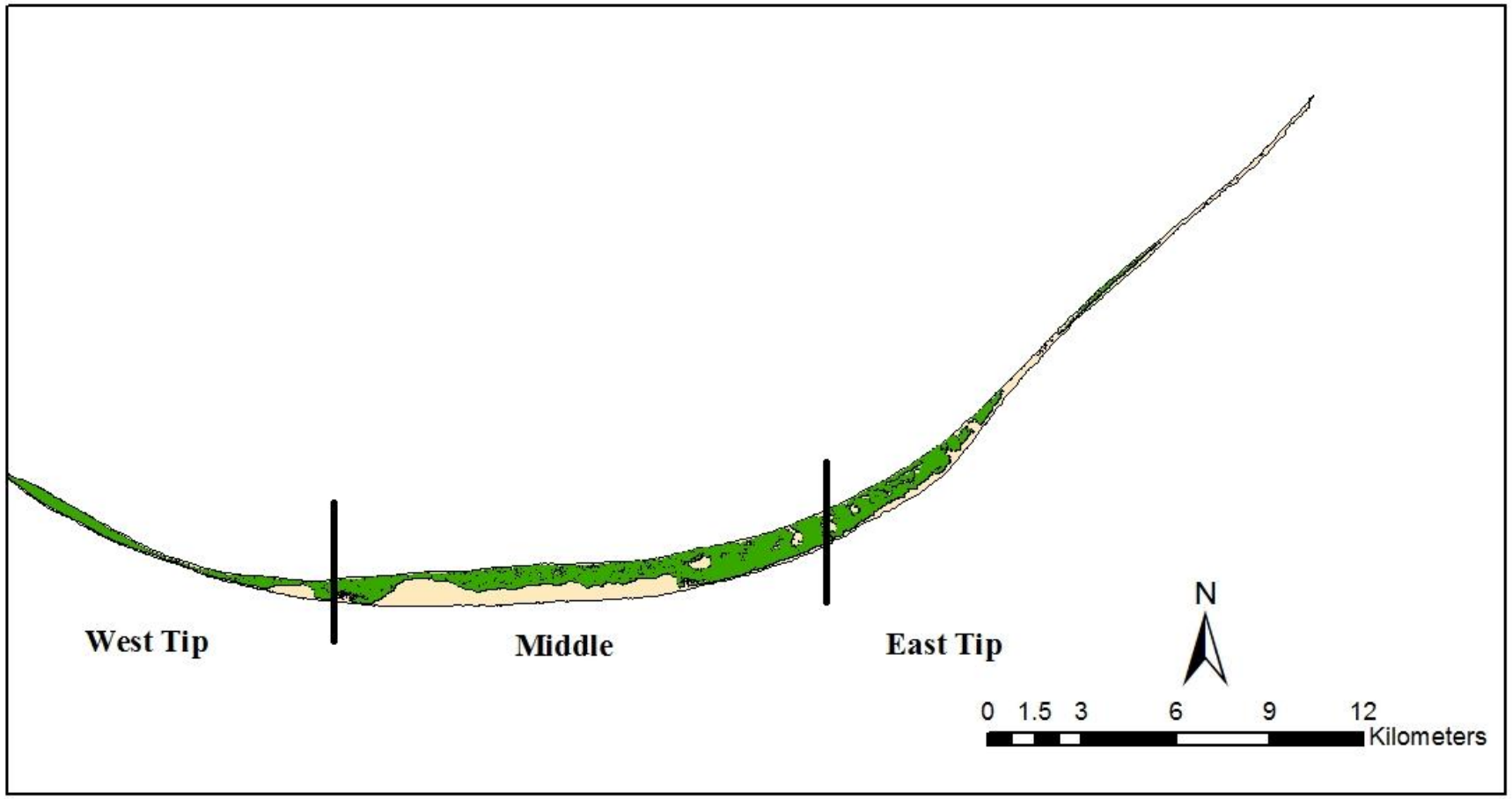


**Figure 2.3**: Map of Sable Island, Nova Scotia, showing the island's major divisions in which area-specific attributes and horse $\delta^{15}N$ were statistically compared.

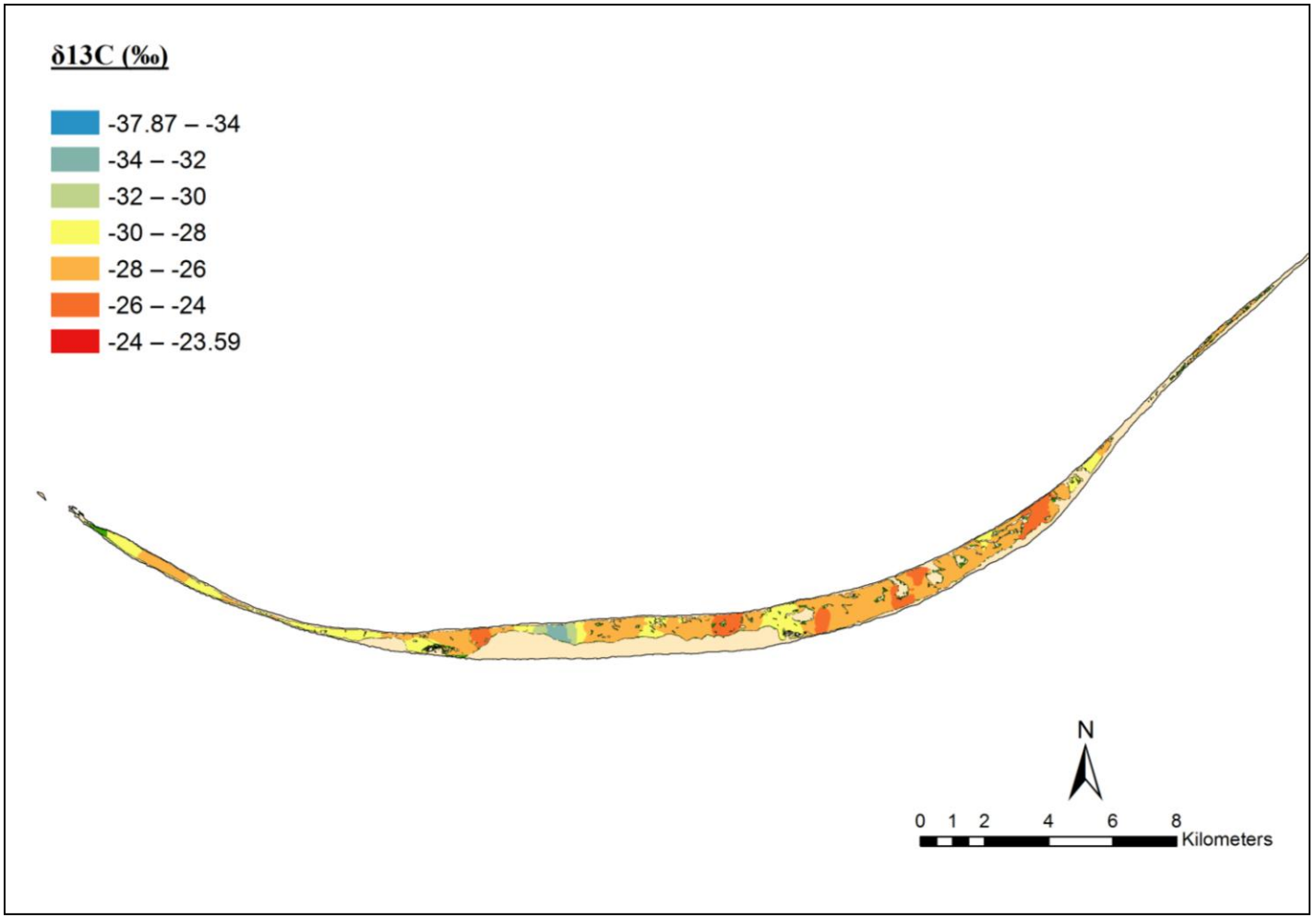


**Figure 2.4**: Ordinary kriged surface ($n$ = 282, RMS = 1.29, Average Std. Error = 1.26) of the distribution of marram grass $\delta^{13}C$ values from sampled sites. More enriched sites are represented by warmer colours which are indicated by the legend. Unvegetated areas are displayed in grey. Marram $\delta^{13}C$ values ranged from -37.87 to -23.59 ($\mu$ = -27.3, SD = 1.85). The lack of spatial trends and explanatory covariates indicated $\delta^{13}C$ was not to be used in identifying areas of vector-nutrient-transference.

# 3 RESULTS

## 3.1 THE MARRAM $\delta^{15}$N ISOSCAPE

Simple linear regressions indicated strong environmental gradients for: distance from vector species colony, seal density, distance from shoreline, distance from vegetation limit, and species richness (Table 2.1). Significant gradients were found in marram cover and browse within the plots (Table 2.2). Elevation and slope variables were included in the GLM modeling procedure since they are well known from the literature to effect isotopic distributions (Wassenaar et al. 2009).

The distribution of marram $\delta^{15}$N was significantly explained by the distance to colony and the interaction between distance to colony and distance to shoreline which accounted for an $r^2 = 0.4$ ($F_{3,278} = 61$, $P < 0.001$; Table 3.1 ). The effect size for distance to colonies explained almost all the variation within $\delta^{15}$N with significantly higher values in closer proximity to seal colonies. Sites that were farther from a seal colony (~35 m) possessed slightly higher $\delta^{15}$N values the further they were situated from the shoreline. Therefore, the interaction between both variables describes significantly higher $\delta^{15}$N occurring along the edges of the vegetation where the largest seal colonies reside, and slightly higher values in the middle of the island. Both variables were incorporated into the $\delta^{15}$N isoscape model using co-kriging methods.

The final isoscape model was produced from numerous iterations and model parameter adjustments to maximize its predictability and residual error (Figure 3.1). Cross-validation results showed a relatively successful fitting of the model with departures within the distribution of residuals ($n = 282$, RMS = 3.515, Average Std. Error = 3.90; Figure 3.5). I believed the model to be sufficiently accurate due to the complexity of interactions, my sample size for the area measured and results from previously constructed isoscapes with similar error terms (Hobson et al. 2009; Bowen 2010; Rascher et al. 2012). A larger variation was shown in the distribution of marram $\delta^{15}$N values across the island ranging from -8.2‰ to 15.2‰ ($\mu = 3.4$‰, SD = 4.1). Higher $\delta^{15}$N was concentrated within the east and west tips of the island and along the vegetation edges, which coincided with the seal pup distribution layers (Figures 3.3, 3.4). Over 72% of seal

pups occurred within sites that displayed isotopic ranges above 3‰ with higher valued polygons containing higher densities of seals. Middle sections of the island displayed lower $\delta^{15}N$ values unless they were situated near local ponds where evapotranspiration and ammonification processes are amplified (Farina et al. 2003; Figure 3.2). Tern colony sites (i.e., East Light and Main Station colonies) possessed high plant $\delta^{15}N$ values but with less of a wide-spread effect when compared to plots in the vicinity of seal colonies.

## 3.2 INFLUENCE OF BIOGENIC VECTOR SPECIES

### *3.2.1 WITHIN TERN COLONIES*

PRS™-probe results displayed significantly greater concentrations of $NH_4$ ($Z = 2.309$, $P = 0.028$) within tern colony sites but not $NO_3$ (Figure 3.6). Sites within tern colonies were found to have almost three times greater input of total nitrogen. Although there were no differences in average $\delta^{13}C$ values between tern influenced and non-colonial sites, I found plants within tern colonies to be more enriched in $^{15}N$ ($F_{1,37} = 27.8$, $P = 0.011$). Plots that were closer to colony centroids were highly enriched with marine-derived $^{15}N$ and the localization decreased with further distances ($F_{1,18} = 30.2$, $r^2 = 0.67$, $P < 0.001$; Figure 3.7). There were no differences between sites for total vegetation cover or species richness.

### *3.2.2 VECTOR SPECIES ON PLANTS*

Plots with the influence of colonies possessed significantly higher $\delta^{15}N$ values ($\mu = 5.59$, SD = 3.77) compared to non-colonial ($\mu = 0.79$, SD = 2.6) sites ($F_{1,280} = 142.5$, $P < 0.001$). $\delta^{15}N$ values strongly differed between all vector species ($F_{3,278} = 115$, $P < 0.001$) with seal influenced plots possessing the highest values and non-colonial sites possessing the lowest (Figure 3.8). Plots influenced by horses and terns displayed values within the middle of these two extremes with tern plots displaying slightly more enriched values (Figure 3.8). Total vegetation cover significantly differed between all groups ($F_{3,269} = 26.1$, $P < 0.001$) except between tern-seal influenced plots and tern-horse influenced plots (Figure 3.8).

These results coincided with the $\delta^{15}N$ values from vector species, indicating grey seals as the top consumer followed closely by both species of seabirds (Figure 3.9). Horse $\delta^{15}N$ values resembled marram isotope values which are their primary food source (Figure 3.9). Grey seal isotope values were consistent with records from other studies, ranging from -15‰ to -17‰ for $\delta^{13}C$ and 16‰ to 18‰ for $\delta^{15}N$ depending on the age and sex of the individual (Tucker et al. 2007).

There were no differences in species richness for plots within and outside of colonies. $\delta^{15}N$ values did not change if guano or feces were present within the plot. As indicated during the formation of the isoscape, plots that were closer to colonies possessed higher $\delta^{15}N$ values ($F_{1,280}$ = 158.2, $P < 0.001$; Figure 3.10). Local seal densities also had a positive effect on $\delta^{15}N$ values ($F_{1,280}$ = 85.9, $P < 0.001$; Figure 3.11).

### *3.2.3 INDIRECT EFFECTS OF VECTORS ON HORSES*

Results from the ANOVA indicated horses located within the tips of the island were more enriched in $^{15}N$ ($F_{2,57}$ = 10.7, $P < 0.001$), although horses within the west tip possessed $\delta^{15}N$ values very similar to horses in the middle ($P$ = 0.04; Figure 3.12). This relation was further supported by the results in comparing horse centroids to their distances from either tips ($F_{1,60}$ = 13.4, $r^2$ = 0.18, $P < 0.001$), but the trend was not found for $\delta^{13}C$ (Figure 3.13). Marram $\delta^{15}N$ values were significantly higher in the tips of the island ($F_{1,60}$ = 13.4, $P < 0.001$), leaving the middle of the island ~ 2.5‰ lower (Figure 3.12). There were no significant differences in sectional-specific $\delta^{13}C$ for both horse and marram isotope values. The tips exhibited greater horse densities, seal densities and beach pea cover while maintaining greater perimeter to area ratios (Table 3.2). Total vegetation cover was found to be higher within the tips of the island ($F_{2,87}$ = 9.14, $P < 0.001$) (Table 3.2). Results from the $\delta^{15}N$ mixing model indicated a greater incorporation of colonial-influenced marram within the diets of horses located within the tips (proportion of colonial-influenced marram within diet = 0.39 ± 0.1) compared to the middle (0.07 ± 0.1) of the island (Figure 3.14).

**Table 3.1**: Significance values and parameter estimates for most parsimonious model in predicting $\delta^{15}N$ isotopic distributions from important environmental variables and their interactions (Sable Island, Nova Scotia, 2010). The model was constructed using a GLM and model selection was performed on the maximal regression model using stepwise AIC. The interaction between colony distance and shore distance described sites being further from a seal colony (~35 m) possessing slightly higher $\delta^{15}N$ values the further they were situated from the shoreline.

| **Variable** | **Estimate** | **Std. Error** | ***t* value** | ***P* value** |
|---|---|---|---|---|
| Intercept | 16.23 | 4.57 | 3.55 | < 0.001 |
| Colony Distance | -5.11 | 1.25 | -4.08 | < 0.001 |
| Shore Distance | -0.79 | 0.91 | -0.88 | 0.38 |
| Colony Distance * Shore Distance | 0.52 | 0.24 | 2.19 | 0.03 |

**Table 3.2**: Island characteristics and plot-specific attributes within the three major divisions of the island: the middle, east and west tips (Sable Island, Nova Scotia, 2010). Horse and seal densities (individuals/km$^2$) were calculated based on the total number of individuals (*n*) located within the vegetation layer. Total number of plots with beach pea (*n* = 119) and plots exhibiting browsed beach pea (*n* = 20) were further divided into major island divisions. Differences in total vegetation cover (*n* = 30 plots for each division, mean ± standard error) were also determined.

| **Island Variables** | ***n*** | **West Tip** | **Middle** | **East Tip** |
|---|---|---|---|---|
| Horse Density (horses/km$^2$) | 448 | 34.9 | 19.2 | 43.5 |
| Seal Density within Veg (seals/km$^2$) | 37,436 | 3121 | 1518 | 3093 |
| Perimeter-Area Ratio (km$^{-1}$) | N/A | 10.4 | 3.2 | 8.6 |
| **Plot-specific Variables** | | | | |
| Beach Pea Cover (%) | 119 | 54 | 16 | 22 |
| Beach Pea Browsed Plots | 20 | 16 | 2 | 2 |
| Total Vegetation Cover (%) | 90 | 76.6 ± 3.8 | 49.6 ± 5.1 | 65.6 ± 4.5 |


**Figure 3.1**: Kriged surface ($n$ = 282, RMS = 3.515, Average Std. Error = 3.90) of the distribution of marram grass $\delta^{15}N$ values from sampled sites (Sable Island, Nova Scotia, 2010). $\delta^{15}N$ values were interpolated using an anisotropic semivariogram incorporating second-order polynomial trend removal at a lag distance of 167 m. More enriched sites are represented by warmer colours which is indicated by the legend. Unvegetated areas are displayed in grey. Marram $\delta^{15}N$ values ranged from -8.25 to +15.18 ($\mu$ = 3.41, SD = 4.03). Higher $\delta^{15}N$ values are concentrated along the east and west tips of the island and along the vegetation edges while middle sections displayed lower $\delta^{15}N$ values.

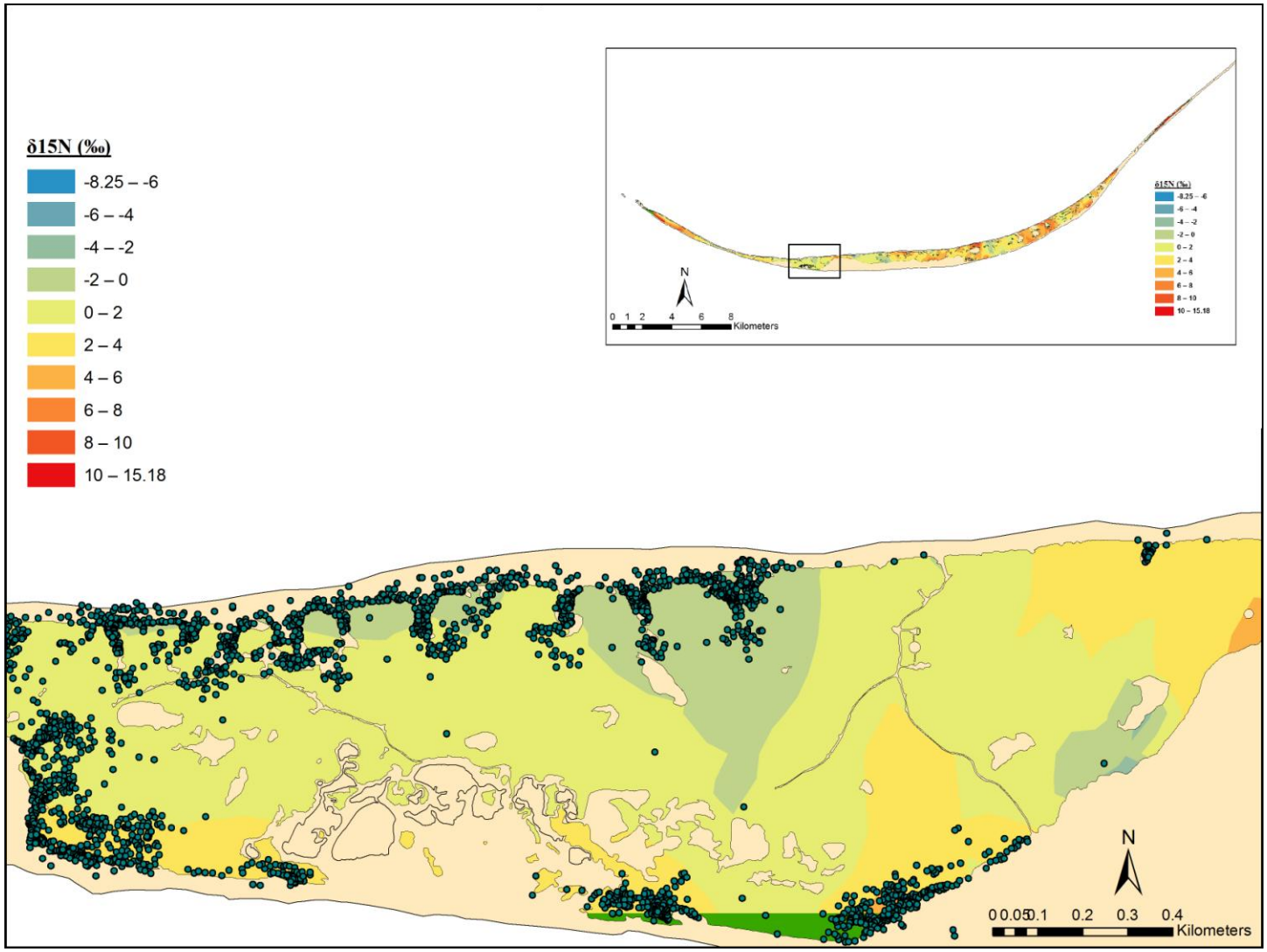


**Figure 3.2:** Snapshot of the $\delta^{15}N$ isoscape within the middle section of the island (Sable Island, Nova Scotia, 2010). Points represent individual seal pups. The middle characteristically exhibited lower $^{15}N$ due to lessened permeability for seal colonies. Unvegetated areas are represented by beige.

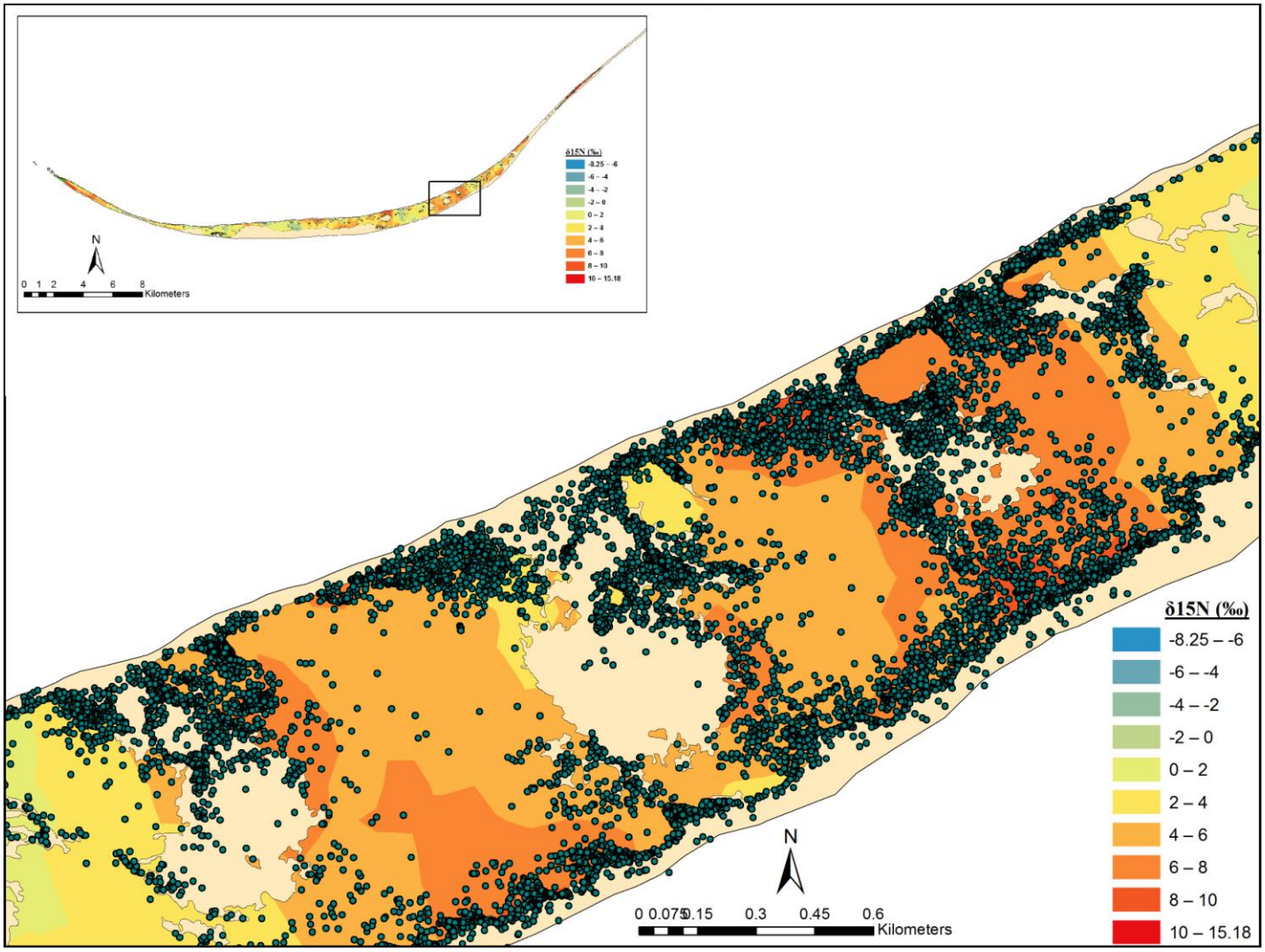


**Figure 3.3:** Snapshot of the $\delta^{15}N$ isoscape within the east tip of the island (Sable Island, Nova Scotia, 2010). Points represent individual seal pups. Tips characteristically exhibited higher enrichment due to greater permeability for seal colonies. Unvegetated areas (i.e., beige) within the middle of the island represent the three bald dunes.

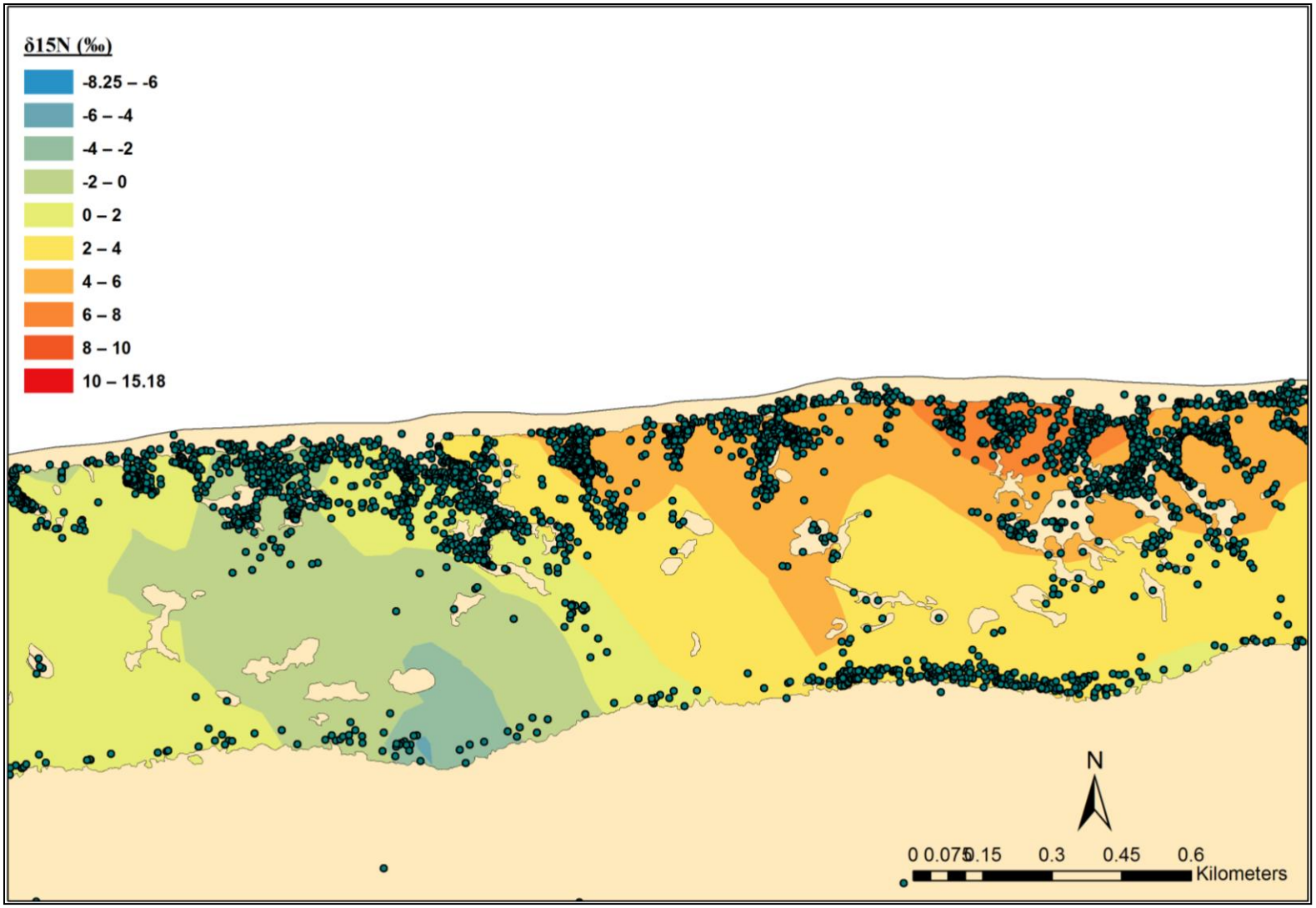


**Figure 3.4:** Snapshot of the $\delta^{15}N$ isoscape within the middle of the island where disturbance by saltation was greatest (Sable Island, Nova Scotia, 2010). Points represent individual seal pups. Greater $^{15}N$ within the middle of the island may be due to the transfer of nutrient rich sand by saltation or ephemeral seals. Unvegetated areas are represented by beige.

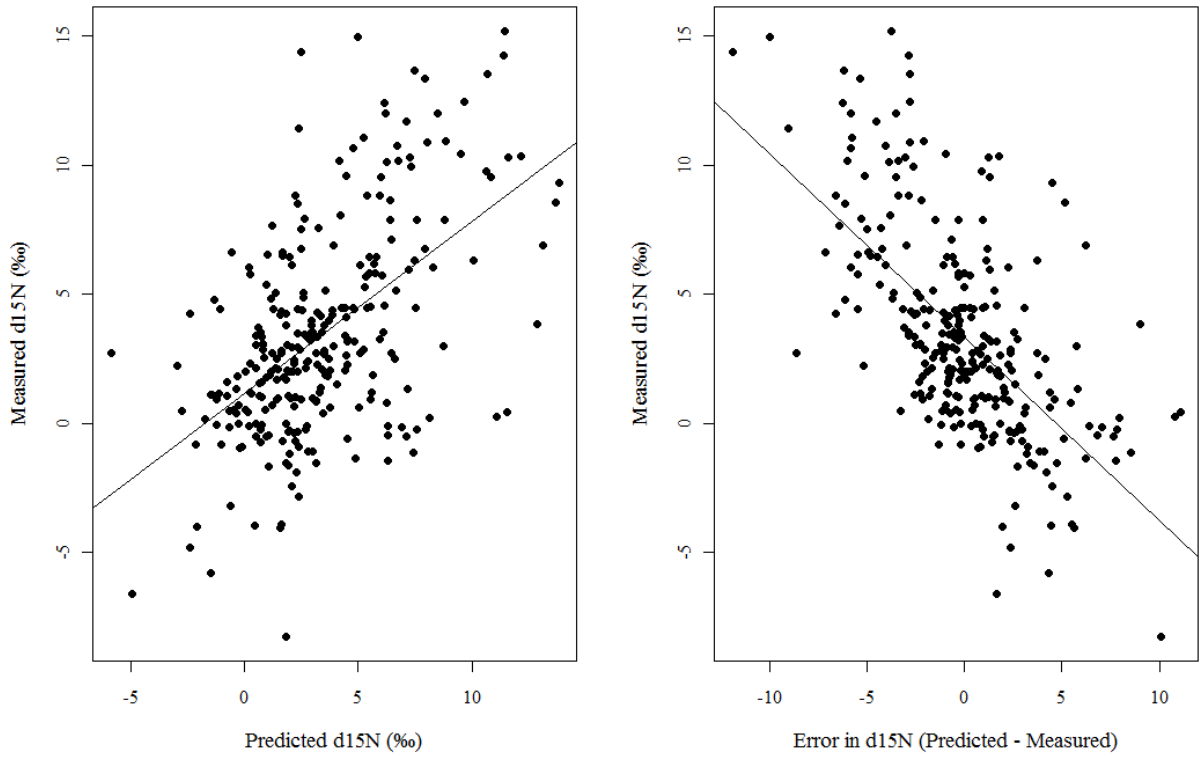


**Figure 3.5**: Regressions between observed values ($n$ = 282) and GLM model predictions ($r^2$ = 0.39) as well as the represented error ($r^2$ = 0.3) within the $\delta^{15}N$ values for the isoscape (Sable Island, Nova Scotia, 2010). Covariates included within the model were distance to shore (m) and distance from seal colony (m).

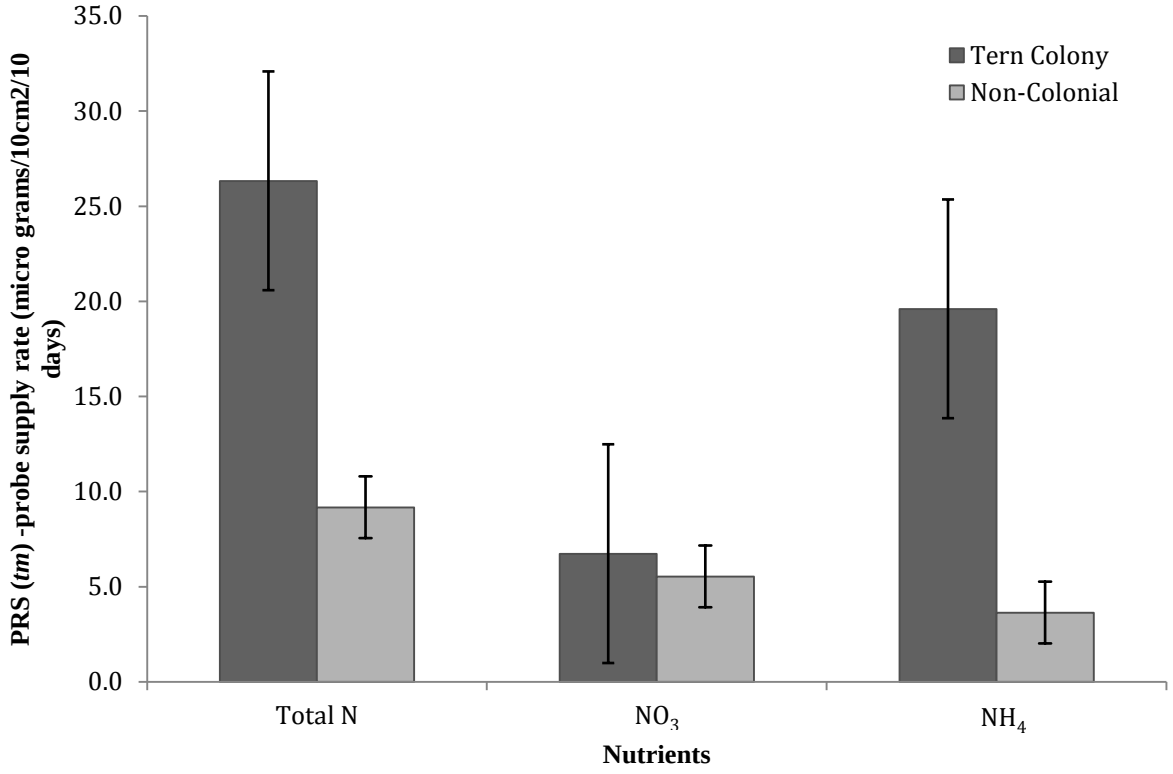


**Figure 3.6:** PRS™-probe data indicating the mean concentrations and standard errors (±) of total soil nitrogen, nitrate and ammonium between tern colony (i.e. East Light and Main Station colonies; $n = 6$) and non-colonial ($n = 6$) plots (Sable Island, Nova Scotia, 2010). PRS™-probes within tern colony sites contained significantly higher total N and $NH_4$ ($Z = 2.309$, $P = 0.028$).

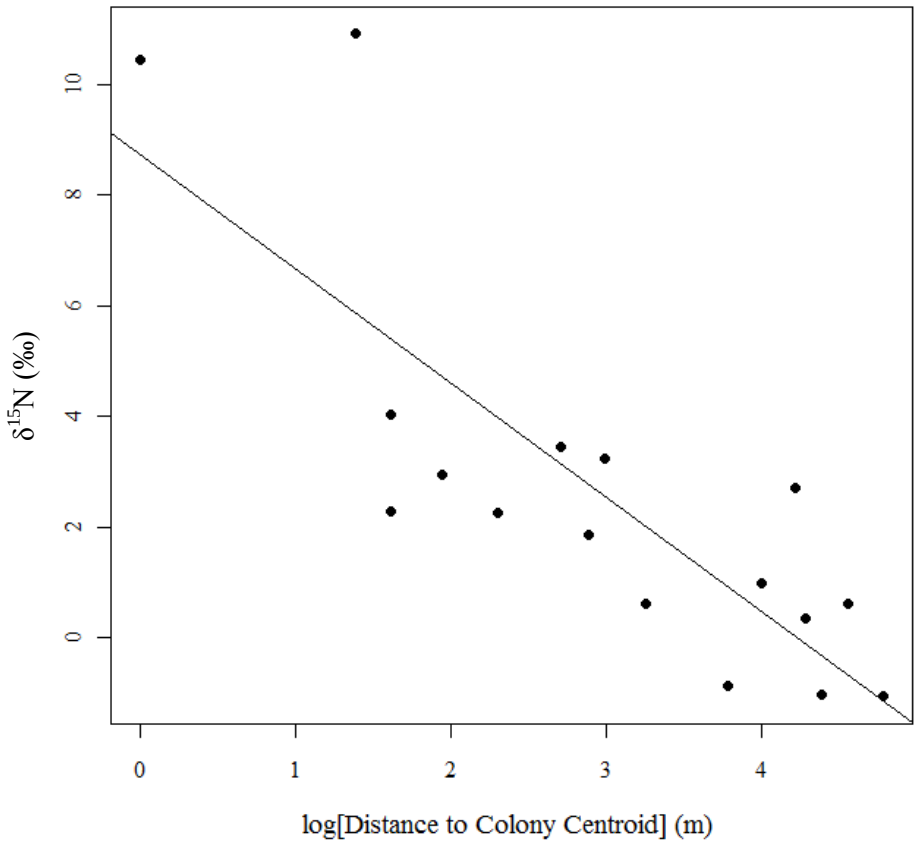


**Figure 3.7:** Linear regression ($r^2$ = 0.67) for the relationship between distance to tern colony centroids (i.e., Main Station and East Light colonies) and marram plant $\delta^{15}N$ values ($n$ = 17) for tern-influenced plots (Sable Island, Nova Scotia, 2010).

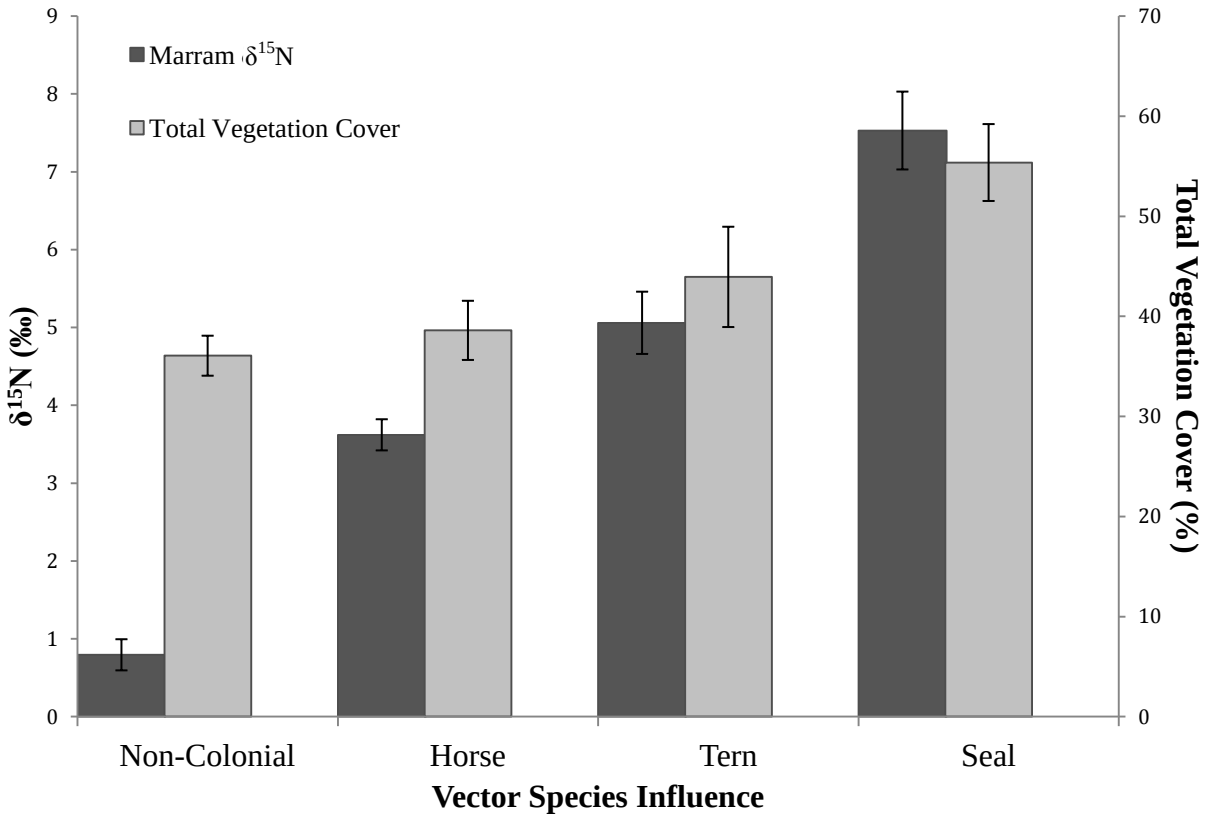


**Figure 3.8:** Means (± standard error) of marram $\delta^{15}$N values ($n$ = 282) categorized by their influence (or lack thereof) from specific biogenic vector species (Sable Island, Nova Scotia, 2010). Mutually-exclusive categories were based on plot-specific attributes, such as the presence of feces within plots or their distance to vector colonies. Vector-influenced plots consisted of those with non-colonial influence ($n$ = 125), horse influence ($n$ = 66), tern influence ($n$ = 27) and seal influence ($n$ = 64). Total vegetation cover plots ($n$ = 217) were divided into non-colonial ($n$ = 102), horse influenced ($n$ = 51), tern influenced ($n$ = 18) and seal influenced ($n$ = 46). Plots with the influence of colonies possessed significantly higher $\delta^{15}$N values ($\mu$ = 5.59, SD = 3.77) compared to non-colonial ($\mu$ = 0.79, SD = 2.6) sites ($F_{1,280}$ = 142.5, $P < 0.001$). $\delta^{15}$N values strongly differed between all vector species ($F_{3,278}$ =115, $P < 0.001$) with seal influenced plots possessing the highest values and non-colonial sites possessing the lowest. Total vegetation cover significantly differed between all groups ($F_{3,269}$ = 26.1, $P < 0.001$) except between tern-seal influenced plots and tern-horse influenced plots.

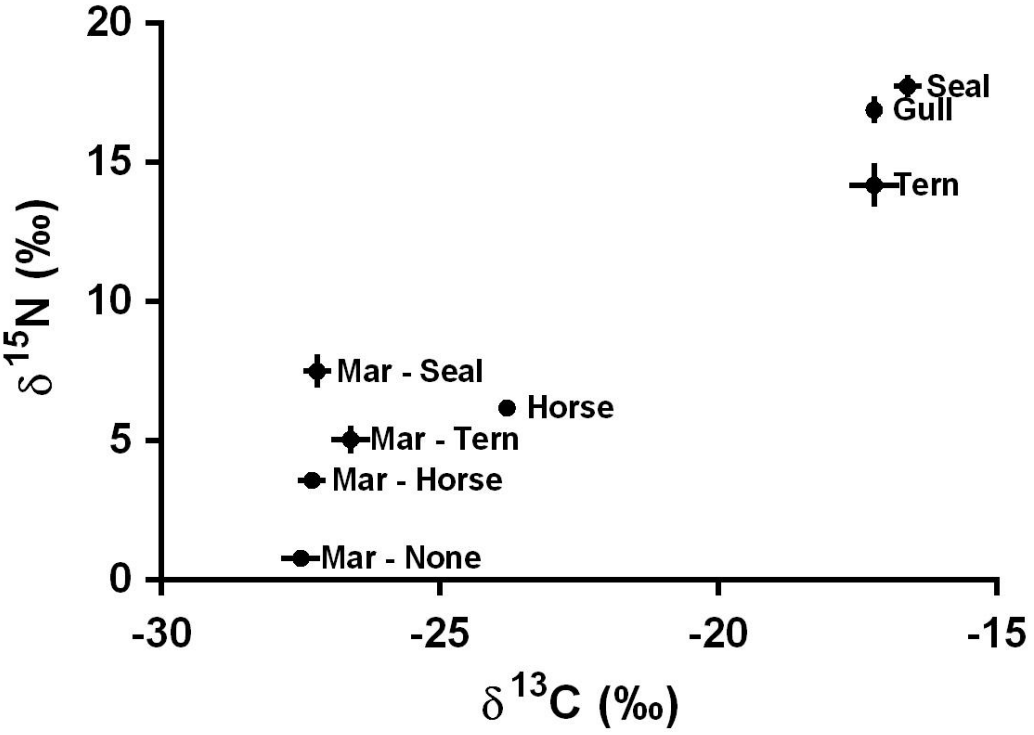


**Figure 3.9:** Stable isotope $\delta^{13}C$ and $\delta^{15}N$ bi-plot displaying means (± standard errors) for marram grass (*n* = 282) influenced by biogenic vector species including horses (*n* = 60), seals (*n* = 14), terns (*n* = 20) and gulls (*n* = 20) (Sable Island, Nova Scotia, 2010). Mean isotope values for vector species are plotted to show potential sources of enrichment.

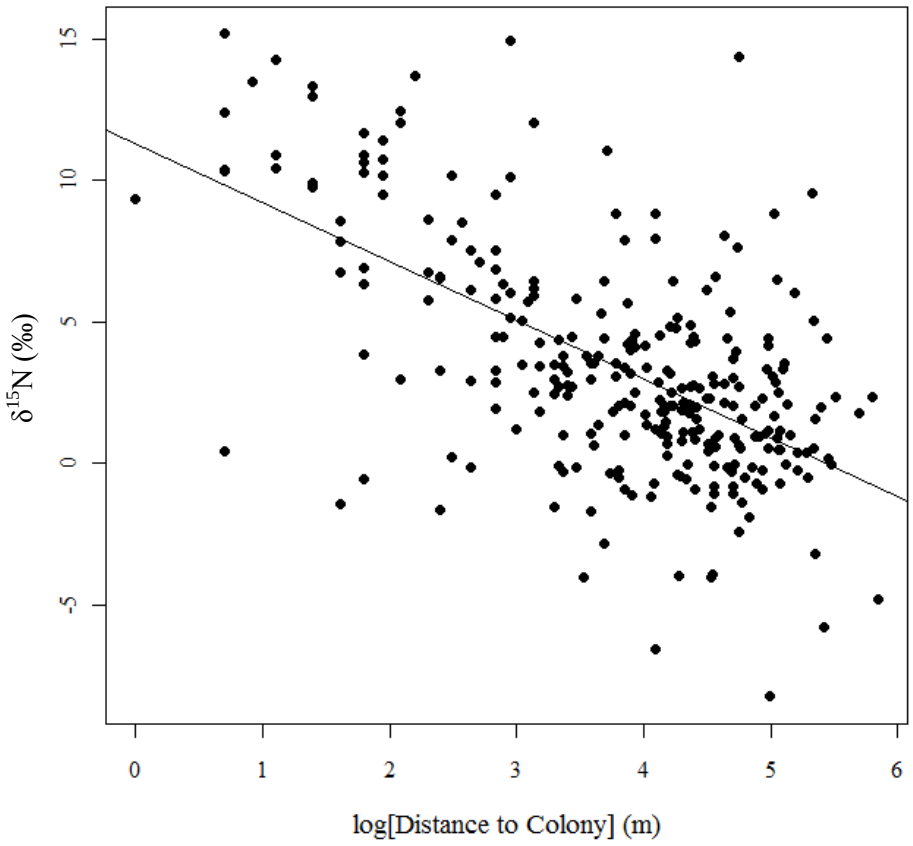


**Figure 3.10:** Linear regression for the relationship between distance to vector colony (log) and marram $\delta^{15}N$ values ($n$ = 282) within individual plots (Sable Island, Nova Scotia, 2010). Colonies were defined as either ≥5 pups within a 15 m radius or as an established tern colony.

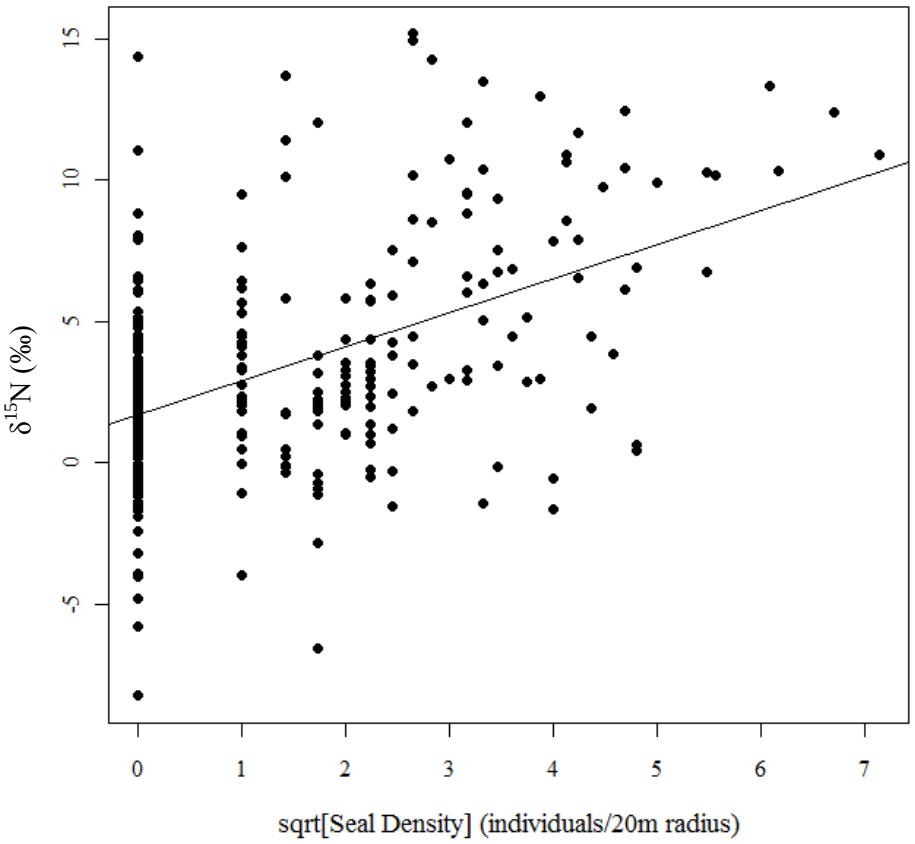


**Figure 3.11:** Linear regression for the relationship between marram plant $\delta^{15}N$ values ($n$ = 282) and local seal densities (square-root) within 20 $m^2$ buffers surrounding individual plots (Sable Island, Nova Scotia, 2010).

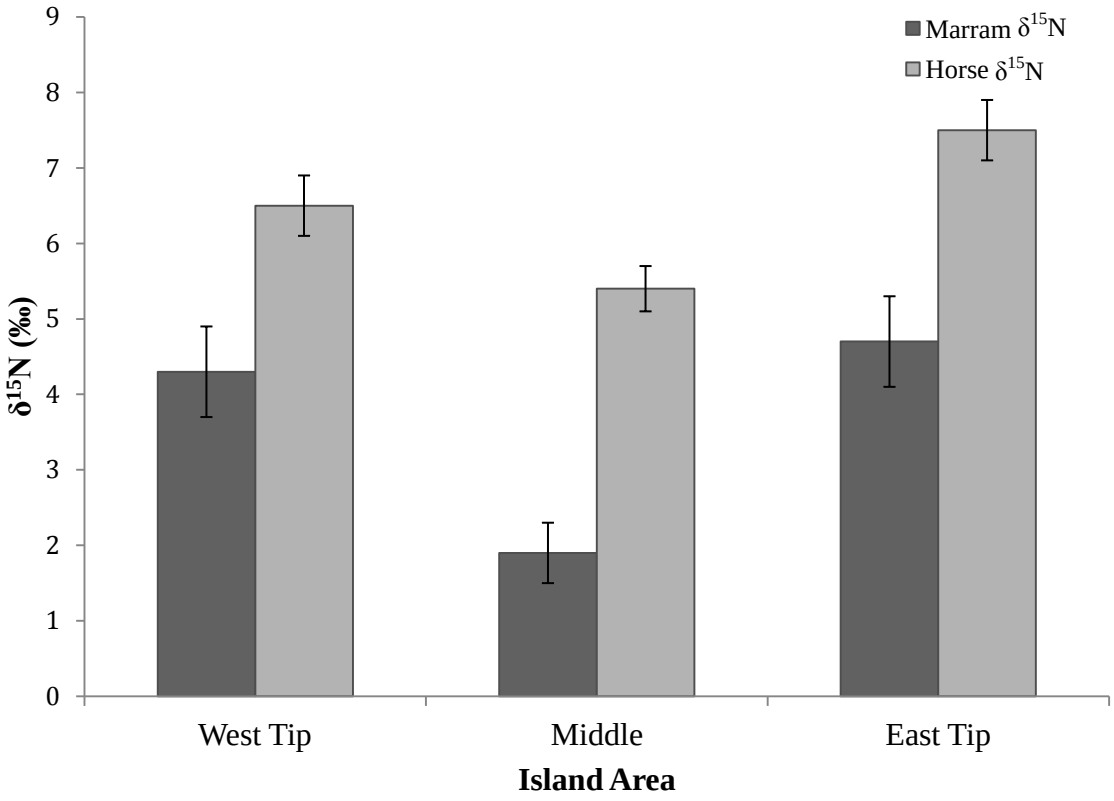


**Figure 3.12:** Means (± standard error) for marram ($n$ = 90) and horse $\delta^{15}N$ ($n$ = 60) values across the three major divisions of the island (Sable Island, Nova Scotia, 2010). Tips possessed larger seal densities, greater seal permeability, and higher perimeter/area ratios than the middle of the island. Marram $\delta^{15}N$ values were significantly higher in the tips of the island ($F_{1,60}$ = 13.4, $P$ < 0.001). Total vegetation cover was found to be higher within the tips of the island ($F_{2,87}$ = 9.14, $P$ < 0.001) (Table 3.2).

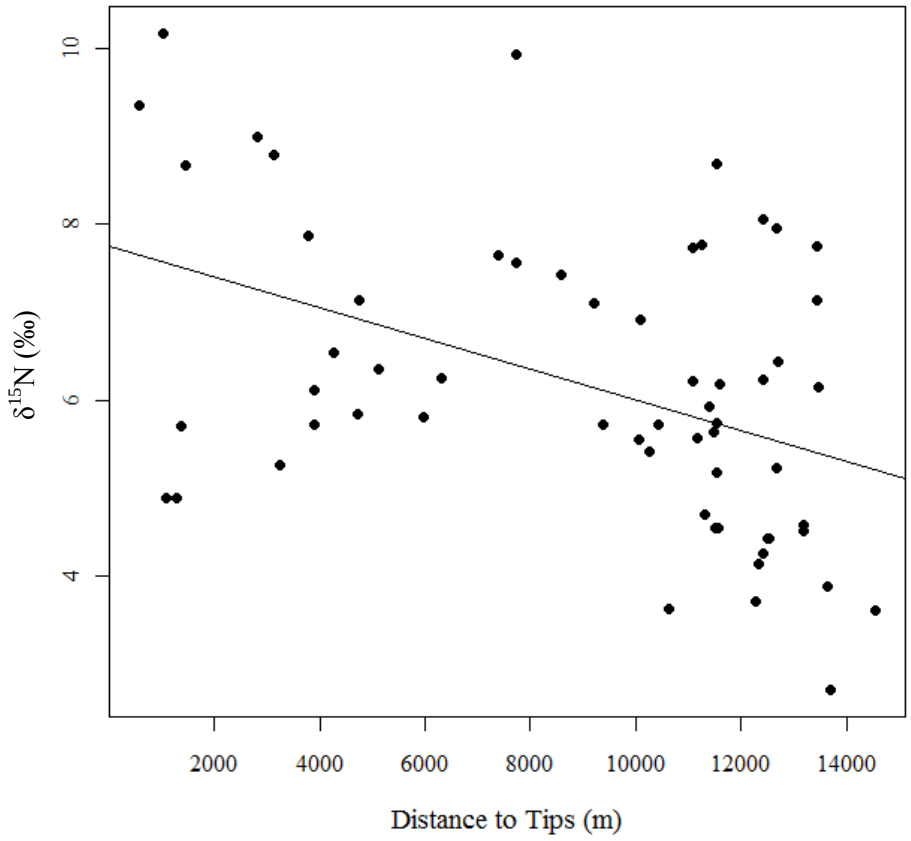


**Figure 3.13:** Linear regression ($r^2$ = 0.18) for the relationship between the distance to island tips (i.e., east and west tips) and horse hair $\delta^{15}N$ values ($n$ = 60) (Sable Island, Nova Scotia, 2010).

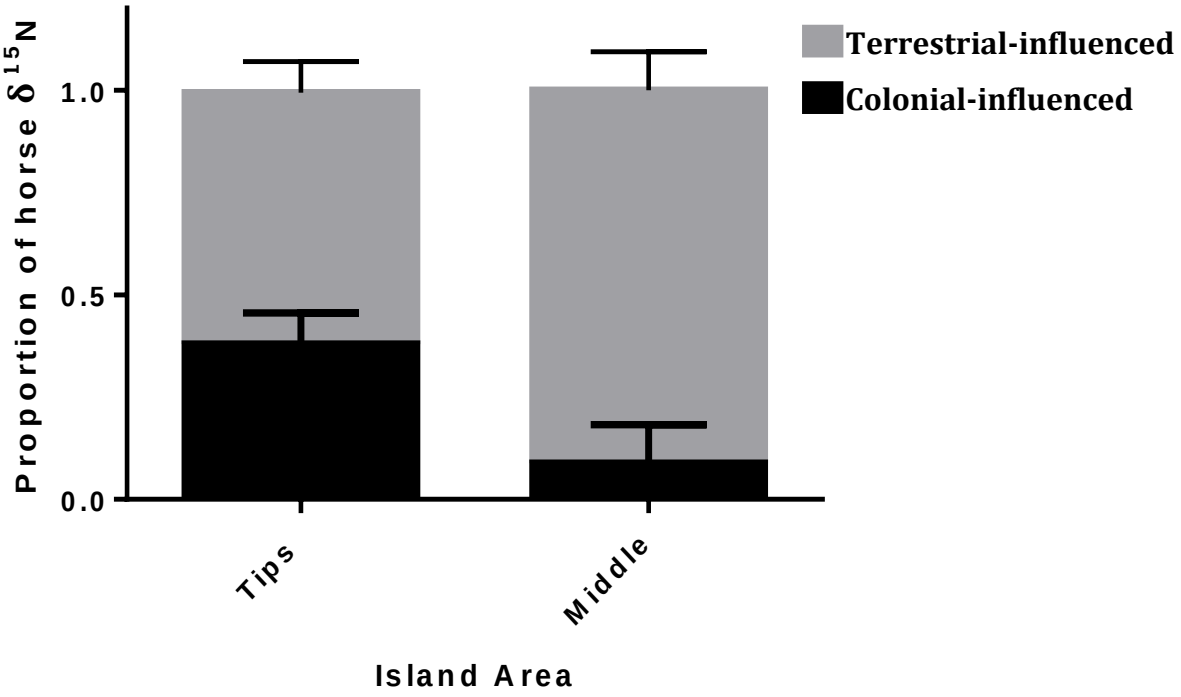


**Figure 3.14:** Results from area-specific $\delta^{15}N$ linear mixing models showing the two source proportional contributions (+ standard error) of terrestrial- and colonial-influenced marram to horse $\delta^{15}N$ within the tips ($n$ = 30) and middle ($n$ = 30) of the island. Both terrestrial- (i.e., horse and non-colonial influenced marram $\delta^{15}N$) and marine-derived (i.e., seal and tern influenced marram $\delta^{15}N$) values were partitioned using IsoError (Phillips and Gregg 2001) incorporating a diet-hair fractionation value of 3.25 ± 0.4‰.

# 4 DISCUSSION

## 4.1 THE MARRAM $\delta^{15}N$ ISOSCAPE

Through co-kriging-interpolation techniques, a plant nitrogen isoscape was created in a first attempt to understand the spatial distribution of $\delta^{15}N$ across Sable Island. This $\delta^{15}N$ isoscape displayed significant geospatial structure in relation to the intensity of biogenic vector activity. Using marram as a means of understanding biogenic vector nutrient transference on the island proved useful due to its abundance across the island (97% of plots) and in displaying great variability for $\delta^{15}N$ (ranging from -8.25‰ to 15.14‰). The lack of spatial variability found in $\delta^{13}C$ is characteristic of other nutrient transference studies, since the values describe macro-trends within entire systems and not local variations (Maguas et al. 1993). Historically, plant $\delta^{13}C$ values have been used to examine local variations in intrinsic water-use efficiency or in understanding trophic hierarchies within distinct environments (Lee et al. 2009). I hypothesize that plant $\delta^{13}C$ values showed little variability at the landscape scale because the processes that govern $\delta^{13}C$ variability (i.e., biogeochemistry and water availability) act more on local scales. At a landscape scale, plant consumption of atmospheric $CO_2$ primarily contributes to greater homogeneity in the distribution of plant $\delta^{13}C$ (Ehleringer and Cerling 1995, Sah and Brumme 2003). Minor intraspecific variations are thereby attributed to plant physiology and water-use efficiency of plants on local scales which I was unable to identify using this model (Lee et al. 2009). Therefore, I anticipated overall fewer variations in marram $\delta^{13}C$ values across the island.

The isoscape visually identified the presence of marine-derived nutrients within the plant community and provided a vital link for tracing the influence of biogenic vector species. The progression from island tips to the middle exhibited the steepest gradients in $\delta^{15}N$ values, moving from greater enrichment (i.e., marine-derived $^{15}N$) to nominal values, respectively (Figures 3.2, 3.3). This enrichment was most likely due to the greater permeability for seal colonies within the tips of island, causing greater variability within the middle of island were permeability was reduced. Both tips exhibited much higher perimeter to area ratios and correspondingly greater seal densities which created greater spatial variability in $\delta^{15}N$ values (Table 3.2). Similar results were found by Farina et al. (2003) who found more seal occupations on islands with higher

perimeter-area ratios. This suggests the local topography spatially restricts the movements of vector species which consequently limits their nutrient permeation into island centres. The distribution and movements of the seals visually matched the presence of high perimeter-foredunes within the centre of the island, with more enriched values originating in low blowouts and extending inland (Figures 3.2, 3.3). The north shore consists of established dunes with high elevations and steep slopes that limit seal permeability to shallow inlets and blowouts (Tissier 2011; Figure 3.4). Although permeability increases on the south shore in these areas, large distances from the vegetation to the shore (ranging from 414 m to 830 m) combined with low foredunes greatly limits accessibility (Figure 3.4). Therefore, the distribution of seals in the middle section of the island is concentrated along the perimeter which corresponds with the higher enrichment of $^{15}N$ within plant tissues. Other allochthonous sources of N, including precipitation and sea spray, display lower $\delta^{15}N$ values (~ 2‰) that would not be attributed to the enrichment within these sites (Bokhorst et al. 2007). Both $NH_4$ (i.e., precipitation) and $NO_3$ (i.e., nitrogen fixation) commonly give $\delta^{15}N$ isotope ratios of 0‰ or less (Peters et al. 1978). Nitrogen fixing plants that inhabit the island possess average $\delta^{15}N$ range of -2 ‰ to 2 ‰ which removes their potential contribution to this effect (Peterson and Fry 1987). Although the two major tern colonies resided on the perimeter their contributions were highly localized with little permeation outside the colony boundaries. This is consistent with numerous seabird nutrient transference studies where nutrient deposition is restricted to the spatial limits of the rookeries (Cocks et al. 1998; Erskine et al. 1998). This means that the intense $^{15}N$ enrichment in these sites can primarily be attributed to the greater seal colony presence and permeability.

Plant $\delta^{15}N$ values within the centre of the island were characteristically $^{15}N$ depleted ($\delta^{15}N <$ 2‰) or slightly enriched (2–4‰) compared to other grass species (Lee et al. 2009). Without continual deposition of nutrients derived from vector species the centre would exhibit N losses similar to other nutrient-deficient environments. The effects of denitrification, volatilization and leaching would be amplified in these areas, contributing to lower residence times for heavier $^{15}N$ within the region; however, some areas within the centre indicate greater resource availability through further enrichment. Following the breeding season ephemeral adult male grey seals and older pups roam the middle of the island, extending beyond more regularly accessible areas and potentially providing additional nutrients to these sites (Noren et al. 2007). At a landscape scale

the continual deposition of marine-derived nutrients at such a wide range enriches the entire terrestrial ecosystem. This effect may counteract the effects of other environmental gradients (i.e., altitude, slope, etc.) on plant $\delta^{15}N$. This slight enrichment may also be attributed to either the transfer of nutrient-rich topsoil or by means of the horse population which exhibit similar isotopic values to their primary food (Schoeninger and DeNiro 1984; Peterson and Fry 1987; Figure 3.9). Sandy environments display greater disturbance of topsoils due to intense saltation and periods of inundation (Tissier 2011). Since Sable Island regularly experiences prevailing north westerly winds, saltation could provide a means by which highly enriched topsoil is transferred southward to more nutrient-poor sites (Bokhorst et al. 2007). However, studies on penguin colonies in the Macquarie Islands suggest the fractionation of marine-derived N from the volatilization of ammonia within colonies results in the airborne transfer of isotopically lighter $^{14}N$ to sites located up to 600 m away (Erskine et al. 1998). The rapid volitization of ammonia within penguin colonies results in the absorption of isotopically light $NH_3$ (i.e., lower $\delta^{15}N$) directly through plant cuticles by the surrounding plant communities. Therefore, the $^{15}N$ depleted-center of the island may be a function of the airborne transfer of isotopically-lighter $NH_3$ from areas of increased seal activity. More analysis is needed to determine what factors contribute to this isotopically-homogenous centre region and how the post-reproductive movement of seals affects the isotopic distribution of $\delta^{15}N$ across the island.

Although distance to colony captured a significant amount of variance in the broad-scaled distribution of $\delta^{15}N$ values across the island, it is anticipated that more complex patterns exist. I expected a greater disparity in $\delta^{15}N$ and $\delta^{13}C$ values between numerous environmental variables, including vegetation community types, presence of feces and plot position; however, the complexity of interactions between these variables dramatically increases with greater spatial scales. The incorporation of numerous landscape-specific variables (i.e. distance to water, LIDAR elevation/slope maps, etc.) may improve model efficiency, although further research is needed into determining these variables. Since the island exhibits different spatial trends at the tips, the construction of sub-regional isoscapes (i.e., tips vs middle of the island) might be more appropriate to improve individual model performance by incorporating local variations in topography (Hobson et al. 2009). In the attempt to model sub-regional $\delta^{15}N$ distributions the regression results indicated better model performance at the tips of the island and greater

statistical noise within the middle. This suggests the presence of differing spatial trends, dependent on unexplored measures (i.e., soil water content, sectional horse density, etc.), govern the distribution of $\delta^{15}N$ in the middle of the island.

My results showed no direct relation between elevations or slopes which are common topographical limiters to seal permeability and have been shown to lower $\delta^{15}N$ values through volatilization or ammonification (Craine and Lee 2003; Farina et al. 2003; Mannel et al. 2007). This is attributed to the treating of plots with equal elevations and slopes as having the same permeability to seal colonies. Although plots may appear similar, their accessibility may differ due to numerous site-specific topographical limitations (i.e., distance to shore, inland dunes, blowouts, etc.) that serve as impassable barriers to permeability. Since there is a high degree of variability in the local topography of Sable Island, future work will involve creating a functional seal permeability index that incorporates transitional topographical limitations in accessing individual plots. Horses and gulls, which commonly graze and nest in high elevations, may be further contributing to these convoluted results. All gull nesting sites recorded in the field were positioned on dunes with elevations >16 m and horse locations from the APEL horse database indicate their extension into high dunes. In regards to the lack of difference found across slopes, I postulate that the forces of saltation and erosion are more important in sandy environments due to a lessened cohesion between sand particles. Some areas surrounding dunes situated in the centre of the island (i.e., bald dunes) exhibited intense enrichment (Figure 3.3). This may be attributed to the greater local density of seals or the greater evapotranspiration and ammonification rates associated with these low water-accumulating areas. Lighter $^{14}N$ would most likely be volatilized into the atmosphere leaving heavier $^{15}N$ within the soils surrounding these dune sites, thereby increasing plant $\delta^{15}N$ values.

Visualizing the spatial structure of any variable of concern is dependent on the researcher's inputs for the nearest neighbor calculations. When the sample size for plots is at a minimum threshold, decreasing the number of nearest neighbors within the interpolation output can erroneously indicate higher variability across a local gradient. Smoothing and optimization functions can aid in combating spatial patterning errors, but they too can drastically affect the output of a model. Although increasing the sample size of isotopically measured sites within the

model will increase the predictability of the interpolation surface, generally feasibility and time constraints can limit exhaustive sampling. Therefore, interpolation surfaces must be developed through numerous iterations and with significant predictor variables to increase the predictability and decrease researcher subjectivity within the final model. This will increase the reliability and applicability of isoscapes in future studies on nutrient transference and interactions. The method used in this study was an improvement over conventional nutrient pathway analyses due to its incorporation of spatial dimensionality present in the marram $\delta^{15}N$ distribution as well as its applicability in tracing broad patterns in nutrient transference (Rascher et al. 2012). Creating large-scaled interpolation surfaces has its limitations, especially in detecting fine-scaled heterogeneity or in accounting for complex interactions common to ecological datasets (Stahl et al. 2011; Rascher et al. 2012). However, if the applicable spatial scale is defined and sampled correctly, isoscapes can be a valuable framework for further identifying and understanding these macro-relations on landscape, regional and global scales.

## 4.2 INFLUENCE OF BIOGENIC VECTOR SPECIES

### *4.2.1 WITHIN TERN COLONIES*

Comparisons between tern colony and non-colony sites confirmed terns as an important vector for nutrient deposition and in altering local biogeochemical processes. The greater presence of $NH_3$ within tern colonies provides a richer source of nutrients for plant communities and increases the total soil N concentrations above normal capacities (Figure 3.6). Seabird excreta deposition is by uric acid which rapidly decomposes through soil microbial activity to $NH_3$ or $NH_4$ by volatilization (Mizutani et al. 1986). Since microbial activity is greatly reduced and volatilization is greatly increased in sandy environments, the vast majority of tern deposition is rapidly volatized to $NH_4$ (Adam and Duncan 2001; Mattos Jr. et al. 2003). My results supported findings by Erskine et al. (1998), indicating the greater concentration of $NH_4$ during these peak periods as the major contributor to the overall three times greater N concentration within the soils. Results from isotopic comparisons indicate that these nutrients are then captured and sequestered within the local plant communities, thereby subsidizing plant communities within these nutrient-deficient environments. Although terns are an important nutrient source, further

examination is needed to determine their effects across a variety of different plant communities and topographies.

My results showed no differences in total vegetation cover and species richness within tern colonies, suggesting the lack of deleterious concentrations of nutrients found in other studies (Mizutani and Wada 1988; Cocks 1997; Ellis et al. 2006). There were also no indications of greater disturbance or the maintaining of early successional stages for plant communities seen in other colonial studies (Crait and Ben-David 2007). Since soil acidification is intensified within areas with greater colonial and fecal residence times, both the shorter nesting period and intense sand disturbance might contribute to the lack of intense concentrations (Ellis et al. 2006). This acidification of soils could increase local rates of volatilization and denitrification, limiting the assimilation of soil nutrients into the plants (Erskine et al. 1998). Terns may also have more widespread and less concentrated effects on isotopic distributions due to their greater movement across the landscape. This is supported by my results comparing $\delta^{15}N$ values to the distance from each plot to the colony centroids, which I expected to have a stronger relation due to greater localization of $^{15}N$ (Erskine et al. 1998; Figure 3.7). Terns spend a large proportion of time in-flight foraging along the shorelines and have lower nest densities than their less transitory seabird counterparts (Lindeboom 1984; Mizutani and Wada 1988; Ellis et al. 2006). As a result, terns may possess a broader role in nutrient transference, depositing marine-derived nutrients across ecosystem boundaries due to their more transitory nature. Overall, this translates to less $^{15}N$ enrichment and nutrient inputs in comparison to other seabird studies (i.e., penguin influenced grasses $\delta^{15}N = 15.1 \pm 1.7‰$). Finally, the non-colonial site used to contrast the tern colonies was selected for its similar community structure and local topography which might have limited any further comparisons beyond isotope values. Future research will need to identify the effects terns have on community stability and structure as well as the cation/anion uptake rates within these colonial areas.

If tern colonies provide a non-deleterious source of nutrients within colonial sites then their presence should increase local resource availability above its intrinsic capacity and consequently increase local plant cover. Numerous studies have shown the increase in $^{15}N$ enrichment and total vegetation cover within or around seabird colonies (Mizutani et al. 1986; Erskine et al.

1998). However, nutrient deposition within soil does not indicate its assimilation into the ecosystem, rather its functional use within various biogeochemical processes. Further analysis is needed to examine the variety of factors that play a significant role in nutrient retention times and uptake. In order to determine the direct uptake of soil nutrients within surrounding plant tissues, isotopic tracers can be used at local-scales; although their effectiveness decreases in highly leached soils (Handley et al. 1999). The rate of $NH_3$ leaching or volatilization shows greater heterogeneity in sandy environments and is greatly dependent on a variety of environmental conditions (i.e., altitude, precipitation, etc.) (Praveen-Kumar and Aggarwal 1998). Mizuntani et al. (1986) found $NH_3$ volitization to have a predominant effect on soil $^{15}N$ enrichment within the top 5 cm of surface soils. The high degree of leaching and disturbance in this sandy environment could counteract the marine $^{15}N$ influence by decreasing the residence time of enriched $^{15}N$ within topsoils. Areas with greater plant cover reduce the impact of disturbance and reduce N leaching, consequently increasing nutrient residence times and enriching isotope values (Beckwith et al. 1998). Although more research is needed, I hypothesize that the combination of decreased nutrient retention times and less bio-concentration of tern species contributes to the lack of difference in total vegetation cover.

### *4.2.2 VECTOR SPECIES ON PLANTS*

My results suggest that N dynamics are highly influenced by the presence of biogenic vector species, which provide local $^{15}N$ enrichment of the surrounding vegetation on a scale dependent on the species type and their mobility. All candidate biogenic vector species displayed distinct $\delta^{15}N$ signatures which provided a means to identify source origins or $^{15}N$ signatures within the local plant community (Figure 3.9). I assumed tern and gull $\delta^{15}N$ signatures would be the same considering their similar trophic position; however, the predation of terns by gulls contributed to their slightly higher $\delta^{15}N$ values (Horn and Taylor 2000). Adult tern and gull feather samples resembled other recorded seabird values except for less mobile seabird species (i.e., penguin $\delta^{15}N = 12.3‰$ and $\delta^{13}C = -17.3‰$) (Mizutani and Wada 1988). Horse values were much lower than domestic horse $\delta^{15}N$ values (~ 9.5‰), though this enrichment was primarily caused by differences in diet since consumer tissues exhibit little fractionation from their diets (Schoeninger and DeNiro 1984; Peterson and Fry 1987; Bol and Pflleger 2002). All $\delta^{15}N$

signatures positively displaced non-colonial $\delta^{15}N$ values by increasing local plant $^{15}N$ through the deposition of enriched N. Visually, all vector $\delta^{15}N$ values can be transposed to their corresponding plots, suggesting their influence strongly contributed to the local $\delta^{15}N$ values within the plant community (Figure 3.8). These results were similar to studies examining the contribution of salmon and bear derived nutrients on Alaskan forests which found their contributions to be up to 24% of the riparian budgets (Helfield and Naiman 2006). Influenced plots showed a characteristic trophic shift of ~2‰ increase with increasing trophic positioning of the influencing vector species (McCutchan Jr. et al. 2003; Figure 3.8). Other food-web studies found similar incremental chains in trophic hierarchies, exhibiting a standard 2–4‰ rise in $\delta^{15}N$ with increasing trophic positioning (Erskine et al. 1998; Eggers and Jones 2000). My findings suggest the addition of marine-derived nutrients within these colonial sites may increase the local resource availability above its intrinsic capacity and contribute to the greater spatial variability exhibited on islands with vectors.

Highly condensed distributions of less mobile species (i.e., seals) provide intense periods of nutrient inputs, while more mobile species (i.e., horses and terns) generate a widespread influence (Farina et al. 2003). The positive relationship between seal densities and marram $\delta^{15}N$ values supports this effect since larger colonies tend to be less transitory. Similar to other studies on pinnipeds, larger densities of grey seals were spatially restricted to perimeter shorelines and inlets where the greatest nutrient enrichment occurred. Areas of the island, where seal densities were lowest, occasionally displayed high $\delta^{15}N$ values indicating greater nutrient enrichment without the presence of vector species (Farina et al. 2003; Figure 3.4). My analysis accounted for the distribution of pups across the island; however, further consideration is needed to incorporate ephemeral males which might contribute to isotopic anomalies within the centre of the island. Another factor to consider is that I defined sites as having the influence of seals if a colony occurred < 25m from the plots. Based on this definition and my results for the distances to colony, I assume that in highly disturbed areas of the island the transport of marine-enriched topsoil could contribute significantly to the enrichment of the surrounding communities (Larney 1998). The higher rate of wind erosion provides optimal conditions for the transference of nutrient-rich topsoils across ecosystem boundaries, affecting the structure and resource availability within otherwise biologically isolated areas (Larney 1998; Ellis et al. 2006).

Although seals have a highly localized effect on plant tissue $\delta^{15}N$, this effect indirectly extends beyond the limits of the colony by enriching communities on a broader scale.

I expected the influence of terns on colony $\delta^{15}N$ plant values to be higher than seal influenced sites considering the intense enrichment shown in other seabird colonies (Cocks et al. 1998; Anderson and Polis 1999; Sanchez-Pinero and Polis 2000). However, these studies have primarily focused on reduced-flight or flightless birds, such as cormorants and penguins, which exhibit greater spatial restriction and corresponding higher $^{15}N$ enrichment (Erskine et al. 1998). The density within tern nest sites and their reduced mobility during nesting periods makes their effect on nutrient deposition more localized during the reproductive season; however, following this period individuals leave the two main colonies and disperse over the entire island (Horn and Taylor 2000)**.** The effects of terns and gulls would therefore be more widespread due to the greater area of occupation; however, the enrichment shown by other highly mobile seabirds was much greater ($\delta^{15}N$ + 10‰) than what was shown in tern-influenced sites (Sobey and Kenworthy 1979; Cocks et al. 1998; Anderson and Polis 1999). This may indicate that seal colonies have a much greater influence than tern colonies in providing marine-derived nutrients onto Sable Island. It is possible that our sampling period limited the effect of terns on the plant communities by not accounting for the time it took for nutrient uptake and tissue synthesis. The intense temporal localization of nutrients during the reproductive periods of seals and terns (i.e., two month time frames) suggests corresponding peaks of nutrient deposition and sequestering within the plant communities (Nakano and Murakami 2001). However, the effects of these nutrient pulses within sandy ecosystems can vary due to various environmental factors (e.g., precipitation, sand deposition, etc.) and are rarely immediate (Polis et al. 1997). Due to the intense leaching and disturbance on the island, I assume that plants compensate by increasing the rate of nutrient uptake and tissue synthesis during these peak periods. Since I sampled during the seeding season for marram (i.e., July–August), I believe the sampling period captured the optimal period of nutrient sequestration for the local plant community.

My results indicated a slight increase in $^{15}N$ enrichment within horse influenced plots, which were identified by the presence of feces or by being in close proximity to horse trails. I concluded that horses create greater N homogeneity within the centre of the island by

redistributing marine-derived N to sites that were naturally prone to greater leaching because of the local topographies (e.g., lower elevations, greater disturbance, etc.). Horses therefore, act as an important terrestrial conduit for nutrient availability within these nutrient-deprived communities due to their greater mobility across ecosystem boundaries (Ellis et al. 2006). Studies by Hilderbrand et al. (1999) determined 15.5–17.8% of the total N in forests within 500 m of Alaskan spawning streams was attributed to brown bear distributed salmon-derived nutrients. This increase in local resource availability could have positive secondary consequences on the community structure and dynamics, thereby promoting ecosystem stability (Huxel et al. 2002). Nutrient deposition by grazers primarily occurs through waste since the effects of carrion are highly localized except for detritivore species (Sanchez-Pinero and Polis 2000). Horse nutrient supplementation primarily occurs through urine deposition which is rapidly converted to $NH_4$ for plant assimilation. Sites with high grazer activity may accelerate nutrient cycling by increasing N mineralization rates that promote plant growth and stability (McNaughton et al. 1997). This results in increasing N availability and retention through greater nutrient deposition as well as further increasing soil microbial activity (Frank et al. 2000). Overall, redistribution of marine-derived N by horses through direct fertilization of soils may create further homogeneity of $\delta^{15}N$ values by supplementing areas that commonly exhibit nutrient deficiency.

Horses may also indirectly govern the distribution of $\delta^{15}N$ across the island through preferential foraging and migrating patterns (Frank and Evans 1997). These factors may change local isotopic distributions if the migration patterns are repeated annually (i.e., removal of isotopically light plants from winter ranges) and if the grazer species consistently follows plant community successions (i.e., targeting nitrogen fixers). Although my results showed no effect of horse browsing, intensive grazing could promote the loss of nutrients through the promotion of $NH_3$ volitization and increased leaching by decreasing plant coverage (Frank and Evans 1997). It has also been suggested that the local horse community limits dune succession by increasing disturbance regimes and contributing to dune instability (Beson 1998; Stalter and Lamont 2006). Total vegetation cover was higher in sites where horses were present (i.e., browse, horse trails, feces) compared to inactive sites. My results indicate that the deleterious effects of over grazing and horse-mediated disturbance are not legitimate factors to consider on Sable Island due to their limited effects and highly dispersed population. Rather the horse population may promote

community stabilization and resource allocation by redistributing nutrients and balancing trophic dynamics. Other negative grazing effects, such as increased microbial immobilization or increased ammonification, would not dramatically contribute to the loss of $^{15}N$ due to the disturbed nature of the sandy soils (Frank and Evans 1997). The combination of increased vegetation cover across the island and overall low grazer densities may limit the effects of grazing to local scales (Table 3.2).

The presence of vector species had a positive effect on vegetation cover within sampled plots, suggesting the greater N enrichment not only subsidized the local plant community with marine-derived nutrients but also promoted plant productivity (Figure 3.8). Due to Sable Island consisting of highly leached soils that are frequently disturbed and eroded, the persistence of nutrients is limited. Consequently, my results differ from studies performed in undisturbed terrestrial environments where further nutrient enrichment beyond ecosystem capacities resulted in destabilized food web dynamics and reductions to species diversities (Huxel et al. 2002; Crait and Ben-David 2007). Ryan and Watkins (1989) showed the effect of intense enrichment in seabird islands were high guano concentrations greatly limited local vegetation cover. By contrast, my results suggest the contribution of allochthonous nutrients increases total vegetation cover which consequently promotes dune stabilization by limiting disturbance (Hester and Mendelssohn 1990; Day 1996). This moderate input of allochthonous nutrients potentially stabilizes the trophic dynamics on Sable Island by maintaining longer food chains and transitional communities (Huxel et al. 2002). The effect of biogenic vector species in promoting primary productivity has been shown in other nutrient-deficient environments, such as in Floridian sand dunes by sea turtles and in Antarctic soils by numerous seabird species (Lindeboom 1984; Bokhorst et al. 2007; Hannan et al. 2007). Compared to other seal inhabited islands, Sable Island presents fewer topographical restrictions to grey seals in accessing the terrestrial community which contributes to greater plant cover (Smith 1978; Norton et al. 1997, Farina et al. 2003). Although dune stabilization and vegetation cover are governed by numerous processes (i.e., erosion, precipitation, disturbance, etc.) the effect of biogenic vector species on the plant communities might indirectly further ecosystem stability in sandy environments. Overall, my results indicate terns as being less important than grey seals in the transfer of

marine-derived nutrients onto Sable Island due to the grey seals' more widespread spatial expansion and greater N enrichment of plants.

### *4.2.3 INDIRECT EFFECTS OF VECTORS ON HORSES*

I hypothesize that the greater enrichment of horse $\delta^{15}N$ values within the tips of the island (i.e., 1–2‰) is primarily attributed to the greater transfer of marine-derived $^{15}N$ via seal colonies. Biogenic vector species provide enriched nutrients through direct or indirect pathways (Sanchez-Pinero and Polis 2000). Direct pathways occur via carrion and excreta that sustain detritivores and scavenger species while indirect pathways facilitate the transfer of nutrients through fertilization of local plant communities and trophic cascades (Polis et al. 1997). Since indirect pathways promote nutrient enrichment via permeation into trophic hierarchies, their bottom-up effects can generate changes in community structures (Bartz and Naiman 2005). Community types differed between the tips and the middle of Sable Island, with more marram-forb communities in the tips and shrub-heath communities in the middle. Although disturbance plays a fundamental role in community establishment on the island, the transfer of nutrients via seals may indirectly contribute to the continued endurance of these highly disturbed perimeter communities that horses depend upon. Observations and browsing data on horse foraging suggest marram is an important browse species for the horse population. Horse $\delta^{15}N$ values were closely linked to marram values, further suggesting their dietary dependence on this grass species. By incorporating the fractionation for marram assimilation, area-specific horse $\delta^{15}N$ was consistent with marram $\delta^{15}N$ values and sectional seal densities (McCutchan Jr. et al. 2003; Figure 3.12; Table 3.2). Results from the horse $\delta^{15}N$ mixing models indicated the enrichment at the tips was due to the ingestion of enriched marram, an indirect consequence of the greater seal densities within these areas (Figure 3.14). As previously shown, the effects of other abiotic means of nutrient transference (e.g., sea spray, precipitation, etc.) could not have caused this enrichment within the tips since they commonly decrease $\delta^{15}N$ values. Greater permeability within the tips, due to higher P/A ratios, facilitated the greater vegetation cover which horses appeared to select for (Table 3.2).

Similar to other studies on seabird islands, the input of allochthonous nutrients increases nutrient content within plant tissue, consequently providing better forage quality for herbivores (Anderson and Polis 1999; Sanchez-Pinero and Polis 2000; Crait and Ben-David 2007). Horse densities were greatest in the tips which suggest a correlation with the greater forage concentrations or further access to freshwater ponds. The greater forage quality and primary productivity within the tips of the island was assimilated into the horse tissues via guano-enriched plants. I suggest that the distribution of seals and terns on the island directly affects local soil nutrients and plant productivity which indirectly determines consumer abundance (Sanchez-Pinero and Polis 2000). As shown in other studies, this could lead to greater productivity of consumers and subsequent increases in their local abundances (Ryan and Watkins 1989; Polis et al. 1997). Numerous species show greater densities within seabird roosting islands, such as rodents (*Peromyscus* spp.) and spiders (*Filistata* and *Latrodectus*) displaying both 1.6 times and 2 times greater densities (Polis and Hurd 1996; Sanchez-Pinero and Polis 2000). At a local scale, horse densities matched the densities of seals within these vegetated divisions, while at a regional scale both populations have been increasing in overall abundance (Table 1.4). This indicates an indirect linkage between the two species, with grey seals exhibiting donor-control in regards to nutrient transference to the local horse population. The temporal inputs of marine-derived nutrients could affect the behaviours and movement patterns of terrestrial consumer populations. Although causation cannot be determined due to the complexity of the system, horse foraging strategies may be influenced by the temporal availability of nutrients from vector sources. Therefore, the further radiation of enriched nutrients into higher trophic consumers would depend on the continuing of these massive seasonal inputs, potentially affecting local horse fitness. Overall, terns and grey seals indirectly exhibit donor-control over the nutrient dynamics of the island through the input of marine-derived nutrients which has cascading effects on higher level trophic consumers.

The slightly less enriched values exhibited in the west tip of the island can be explained by the distribution of beach pea across the island. The isotopic value of animals that exhibit preferential foraging strategies (i.e., target plant species in seasonal trends) will fluctuate depending on the isotopic signature of the foraged plant. Being an N-fixing species, beach pea characteristically exhibits lower isotope values ($\delta^{15}N$ and $\delta^{13}C$) (Peterson and Fry 1987; Snyder

and Boss 2002). Horses regularly forage on beach pea and our results indicate the degree of foraging increases with its greater coverage (Table 3.2). Since beach pea is browsed more in the west tip of the island, horse $\delta^{15}N$ values would expectedly decrease with the less enriched food source (Figure 3.12; Table 3.2).

### *4.2.4 RECOMMENDATIONS FOR FUTURE RESEARCH*

Although the isoscape model was useful in describing the spatial distribution of marram $\delta^{15}N$, the identification and incorporation of more important landscape-specific predictor variables is essential for greater predictability of marine nutrient inputs. I identified two variables of importance that explained the spatial distribution of $\delta^{15}N$, distance to colonies and distance to shoreline. However, numerous other environmental conditions could affect plant $\delta^{15}N$ values such as local water fluxes, the temporal presence of nutrients within soils, and rates of disturbance by saltation or inundation. Sources from agricultural research and controlled experiments provide a direct measure in understanding how these variables affect isotopic variability (i.e., fractionation rates, temporal persistence, etc.); however, their effects become more convoluted in complex ecosystems. Future research should focus on incorporating various statistical tools that provide greater predicting or explanatory power to isoscape models. This can be accomplished by including modern statistical techniques, such as structural equation modeling, which identifies the variance attributed to intercorrelated explanatory variables and their causal relations to one another (Anderson and Gerbing 1988; Reisinger and Mavondo 2007). Moving beyond direct linear correlations and towards more complex multivariate analyses could isolate relationships and provide a better understanding of the interactions between variables with greater explanatory efficiency (Hobson et al. 2012). Classically, models have been improved by removing 10-20% of the data to be used for post-hoc validation of the final model; however, the randomized removal of 10% of the data resulted in significantly less predictability, suggesting the model possessed the minimal sample size at this scale. Future procedures will improve the models performance by moving beyond simple co-kriging methods and incorporating more accurate predictive general linear models directly into an interpolation surface.

My research demonstrates the transfer of marine-derived nutrients into the marram community via the grey seal population; however, future research is needed to determine whether this trend occurs for numerous plant species on the island. Crait and Ben David (2007) showed the differential uptake of $^{15}N$ enriched nutrients amongst plant species growing in otter latrine sites. Different plant species display isotopic-specificity; therefore, the effect of nutrient deposition by biogenic vector species should be assessed for a variety of plant species. Ideally, this research would coincide with determining the average $\delta^{15}N$ values for all browse species by developing optimal foraging strategies for the wild horse population. If tissue samples are collected from each browse species then a two-isotope (i.e., N and C) multiple-source ($n$ + 1) mixing model could be constructed to determine the proportional contribution of each plant species to the isotopic composition of the horse's tissue (Phillips 2012). By incorporating trophic fractionation factors and measuring the diets at different periods (i.e., seasons) a direct understanding of the differential foraging strategies could be used for future analyses.

Although my findings indicate a correlation between the densities of horses and grey seals on Sable Island, a quantitative approach is necessary to determine if grey seal colonies affect the population distribution of horses through preferential foraging strategies. Temporal stable isotope records can be gained from tissues with longer turnover times (i.e., hair, bone collagen, teeth etc.), thus providing high resolution, long-term dietary histories of individual animals (Ayliffe et al. 2004; Cerling et al. 2004; Passey and Cerling 2002). Research by Ayliffe et al. (2004) determined the turnover time of horse hair was made of three pools: a fast turnover pool ($t_{1/2}$~0.5 days) encompassing ~41% of the isotope signal, an intermediate pool ($t_{1/2}$~4 days) encompassing ~15%, and a slow turnover pool ($t_{1/2}$~140 days) encompassing ~44%. By sectioning the hair and accounting for the represented turnover times a horse-hair isotopic record could be gained and compared with temporal seal densities to determine periods of nutrient pulses and acquisitions by the horse population. The population dynamics of both grey seals and horses could be compared to determine if the distribution of seals influences the temporal distribution of horses on the island. In addition to distributions, measures of horse survivability and fitness could be compared to their isotopic record and the densities of seals to determine the indirect benefits of nutrient supplementation. Gaining an understanding of the temporal acquisition of marine-derived nutrients combined with an examination of the sectional-specific

population growth rates for both species (i.e., grey seals and horses) would be a more quantitative approach then the correlations presented in my research.

# 5 CONCLUSIONS

Isoscapes that predict the spatial distributions of stable isotopes within environments are important tools in understanding the biogeochemical processes that govern nutrient transfer on island ecosystems. My results indicated there were considerable trends within the spatial distribution of marram $\delta^{15}N$ across the island, suggesting the potential for evaluating isotopic origins from distinct allochthonous sources. The interpolation model identified higher $\delta^{15}N$ within the perimeter and tips of the island, which was closely related to seal colony presence. Multiple complex interactions between correlated environmental variables, including low seal density, were believed to be contributing to greater $\delta^{15}N$ heterogeneity within the centre of the island. Although these areas require further model assessment, this isoscape provides an essential framework in identifying important nutrient gateways. Therefore, isoscapes could be valuable in developing future conservation and management strategies due to their ability to identify differing sources of nutrients at a landscape scale.

Biogenic vector species (i.e. seals and terns) serve as important conduits in transferring marine-derived and highly enriched nutrients onto island communities like Sable Island. These species directly affect the vegetation through the deposition of marine-derived nutrients into the surrounding soils. This effect resonates into local plant communities and higher trophic consumers which indirectly exhibit greater enrichment. Unlike other systems where vector species provide deleterious effects, the local grey seal population appears to be enriching the local vegetation community and having widespread effects on plant productivity where permeability is greatest. Seabird colonies (i.e. terns and gulls) exhibit less widespread effects on the local vegetation, but contribute a significant amount of N in the form of $NH_4$ near colony centroids. I hypothesize that the presence of both species may further ecosystem stability on Sable Island by promoting dune stabilization and providing a continual input of highly enriched nutrients.

Although complex geochemical processes (i.e. evapotranspiration, ammonification, etc.) likely govern the structure of the community within the centre of the island, horses may counteract the local effects of leaching and disturbance. The horse population contributes to the

greater homogeneity of isotope values within the centre of the island through the redistribution of terrestrial nutrients. The horse population's greater enrichment within the tips of the island suggests their further importance in being indirect conduits for the transfer of marine-derived nutrients where permeability of biogenic vector species is reduced. Future studies will need to examine if plant and horse $\delta^{15}N$ corresponds with the temporal availability of marine-derived nutrients. Grey seals and seabirds annually visit the island during specific time periods, resulting in highly concentrated pulses of nutrient deposition. Since plant and horse tissues provide a temporal record of their diet, consumer isotopic records can be examined to determine if the sequestering of marine-derived nutrients occurs within the reproductive periods of the biogenic vector species (Hirons et al. 2001; Dalerum and Angerbjorn 2005). I hypothesize that higher $\delta^{15}N$ values will correspond with these nutrient pulses by biogenic vector species, stimulating further N acquisition by plants during these time periods.

These results have important implications in developing future management and conservation strategies for Sable Island. Due to the depleting cod stocks in the Northern Atlantic Ocean, there has been rising demand in reducing the population of grey seals which view cod as a major nutritional source for their diet (Bowen and Harrison 1994). Debate over the continued presence of the horse population on the island has focused on their deleterious effects to the fragile environment, increasing disturbance regimes by limiting vegetation cover. Recently (i.e. 2012), the Government of Canada and Nova Scotia designated Sable Island as the 43$^{rd}$ National Park under the National Parks Act. My research indicates that the biogenic vector species on Sable Island are in fact indirectly governing trophic dynamics through the establishment of nutrient gateways and which may contribute to the stabilization of Sable Island's vegetative community.

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# APPENDIX A: PLOT-SPECIFIC ISOTOPE VALUES

**Appendix A.1**: Locations and isotope values (i.e., $\delta^{13}C$ and $\delta^{15}N$) for all plots where marram grass was sampled and analyzed ($n$ = 282). Plot Id represents the identification number applied to the plot.

| Plot Id | $\delta^{13}C$ | $\delta^{15}N$ | UTM Northing | UTM Easting |
|---|---|---|---|---|
| 1 | -29.17 | 8.54 | 4871945.145 | 729048.2245 |
| 3 | -29.85 | 10.66 | 4871841.795 | 729146.5834 |
| 4 | -29.36 | 0.92 | 4868985.711 | 741950.4935 |
| 5 | -29.49 | 6.52 | 4869099.889 | 742183.4219 |
| 6 | -27.79 | 1.33 | 4869024.541 | 742221.5423 |
| 7 | -29.7 | 2.01 | 4869062.552 | 742287.6657 |
| 8 | -29.23 | 7.64 | 4868880.082 | 742346.5865 |
| 9 | -30.47 | 1.18 | 4869050.904 | 742364.4471 |
| 10 | -30.59 | -0.07 | 4869014.72 | 742378.6314 |
| 11 | -30.27 | 6.04 | 4868880.783 | 742487.219 |
| 12 | -30.91 | -8.25 | 4868966.485 | 742488.9033 |
| 13 | -29.03 | 0.48 | 4868998.481 | 742572.9289 |
| 14 | -29.56 | 2.5 | 4869651.942 | 750148.1826 |
| 15 | -29.84 | -1.09 | 4869368.676 | 750158.081 |
| 17 | -28.5 | 6.6 | 4869615.724 | 750190.5438 |
| 19 | -30.22 | 4.15 | 4869231.558 | 750267.7588 |
| 21 | -28.58 | 1.21 | 4869351.954 | 750304.2028 |
| 23 | -30.78 | 0.25 | 4869195.002 | 750359.9735 |
| 25 | -28.54 | 11.42 | 4869262.66 | 750413.6832 |
| 27 | -29.09 | 4.36 | 4871901.644 | 729301.1384 |
| 29 | -29.82 | 3.41 | 4871862.606 | 729361.9302 |
| 31 | -29.73 | 1.71 | 4871760.934 | 729540.5615 |
| 33 | -29.05 | 4.26 | 4871766.466 | 729603.8256 |
| 35 | -27.88 | 4.35 | 4871577.197 | 729821.653 |
| 37 | -29.51 | -0.34 | 4871503.713 | 729947.1069 |
| 39 | -27.84 | -0.25 | 4871344.919 | 730177.5492 |
| 41 | -28.23 | 1.95 | 4874293.799 | 759951.9264 |
| 43 | -29.71 | 2.94 | 4874083.707 | 759842.8933 |
| 44 | -29.47 | 2.85 | 4874304.713 | 759833.4044 |
| 47 | -30.3 | 1.34 | 4873330.776 | 759167.8419 |
| 48 | -30.68 | 2.97 | 4868714.464 | 750124.1121 |
| 50 | -27.94 | 0.93 | 4869654.352 | 750094.2386 |
| 52 | -28.04 | 5.8 | 4869165.565 | 749934.2507 |
| 54 | -29.83 | 3.05 | 4869243.8 | 749855.7386 |

| Plot Id | $\delta^{13}C$ | $\delta^{15}N$ | UTM Northing | UTM Easting |
|---|---|---|---|---|
| 56 | -28.7 | -0.29 | 4869154.749 | 749824.534 |
| 58 | -29.42 | 3.53 | 4869315.27 | 749745.3311 |
| 60 | -28.87 | 11.67 | 4869403.119 | 749685.7517 |
| 62 | -28.93 | 7.51 | 4871968.945 | 756509.0334 |
| 64 | -27.99 | -1.91 | 4871893.32 | 756595.5393 |
| 66 | -26.92 | -0.39 | 4871365.814 | 756704.3991 |
| 68 | -27.69 | 2.08 | 4871368.295 | 756653.6722 |
| 70 | -27.55 | 2.74 | 4871636.613 | 756200.4678 |
| 72 | -30.09 | 1.18 | 4871767.477 | 756132.7195 |
| 74 | -28.68 | 4.55 | 4871093.868 | 756117.0024 |
| 76 | -28.64 | 4.45 | 4868756.554 | 736346.4084 |
| 78 | -28.52 | 2.72 | 4868859.206 | 736386.9473 |
| 80 | -29.37 | 2.43 | 4868913.046 | 736555.4048 |
| 82 | -31.17 | 1 | 4868947.039 | 736821.8144 |
| 84 | -27.62 | 3.97 | 4868891.525 | 746213.1782 |
| 86 | -27.64 | 3.99 | 4869038.776 | 746045.3372 |
| 88 | -29.42 | 9.76 | 4869289.001 | 745984.5989 |
| 90 | -28.74 | -0.22 | 4869113.164 | 745802.2427 |
| 92 | -28.12 | -0.12 | 4868825.242 | 745716.4725 |
| 94 | -25.53 | -1.09 | 4869120.188 | 745691.8644 |
| 96 | -31.53 | 7.1 | 4868747.814 | 745638.1593 |
| 98 | -28.07 | 9.52 | 4869209.578 | 745612.9937 |
| 100 | -27.22 | 6.46 | 4868906.925 | 745609.7512 |
| 102 | -29.62 | 9.93 | 4868766.816 | 750479.0595 |
| 104 | -27.49 | 1.68 | 4868924.501 | 750589.6564 |
| 106 | -29.14 | 1.59 | 4869218.507 | 750628.3687 |
| 108 | -28.07 | 4.18 | 4869240.735 | 750657.2691 |
| 110 | -29.45 | 13.51 | 4869785.582 | 750648.5759 |
| 112 | -29.21 | 5.93 | 4869014.704 | 750886.0831 |
| 114 | -29.37 | 2.49 | 4868778.851 | 736876.8549 |
| 116 | -28.96 | 3.78 | 4868727.559 | 737059.5234 |
| 118 | -27.08 | 1.07 | 4868771.551 | 737573.1235 |
| 120 | -28.72 | -0.7 | 4868559.199 | 738472.0894 |
| 122 | -26.51 | -0.91 | 4868715.738 | 738742.1317 |
| 124 | -30.76 | 2.87 | 4868337.811 | 738809.6088 |
| 126 | -28.16 | 4.44 | 4868582.4 | 738899.6507 |
| 130 | -27.71 | 1.55 | 4868698.863 | 739352.7765 |
| 132 | -27.39 | 1.15 | 4868615.913 | 739457.851 |
| 134 | -28.93 | -1.67 | 4868909.496 | 739856.3289 |
| 136 | -26.34 | 0.52 | 4871826.179 | 756843.2432 |
| 140 | -26.83 | 3.55 | 4872027.204 | 756949.5486 |

| Plot Id | $\delta^{13}C$ | $\delta^{15}N$ | UTM Northing | UTM Easting |
|---|---|---|---|---|
| 142 | -29.65 | 2.27 | 4872109.578 | 757008.2247 |
| 144 | -26.56 | 3.08 | 4871778.217 | 757040.3837 |
| 146 | -26.61 | 1.49 | 4871842.613 | 757123.0646 |
| 148 | -28.52 | 4.48 | 4869318.02 | 746495.5165 |
| 150 | -27.69 | 4.1 | 4868963.296 | 746587.4947 |
| 152 | -26.78 | 1.02 | 4868947.18 | 746781.8134 |
| 154 | -27.04 | 4.44 | 4868896.076 | 746872.9418 |
| 157 | -29.07 | 0.8 | 4868757.667 | 747098.3534 |
| 159 | -28.44 | 3.27 | 4869125.064 | 747345.8791 |
| 161 | -28.46 | -1.14 | 4869210.858 | 747409.3889 |
| 163 | -28.06 | -0.04 | 4869566.605 | 752441.4379 |
| 165 | -27.3 | 0.42 | 4869547.4 | 752492.0071 |
| 167 | -27.61 | 2.52 | 4870001.649 | 752502.8156 |
| 169 | -27.59 | 0.71 | 4870118.65 | 752595.6071 |
| 171 | -27.17 | 3.71 | 4869884.776 | 752617.3914 |
| 173 | -27.32 | 3.3 | 4870215.398 | 752624.0651 |
| 176 | -27.92 | -1.56 | 4869652.195 | 733505.9758 |
| 178 | -27.6 | 3.5 | 4870056.539 | 732355.5767 |
| 181 | -28.58 | 3.05 | 4870329.327 | 732111.3939 |
| 182 | -29.55 | 1.85 | 4870235.15 | 731899.3697 |
| 184 | -29.85 | 4.46 | 4870278.243 | 731764.4872 |
| 186 | -34.46 | 2.67 | 4868899.075 | 742591.8418 |
| 188 | -31.58 | 1.06 | 4868826.894 | 742776.942 |
| 190 | -33.04 | 3.26 | 4868695.611 | 743048.6131 |
| 192 | -34.18 | 0.64 | 4869062.758 | 743112.2983 |
| 195 | -33.05 | -0.69 | 4868639.354 | 743515.2753 |
| 197 | -37.87 | 2.14 | 4868925.76 | 743621.2908 |
| 199 | -27.37 | 0.93 | 4869048.708 | 743637.6653 |
| 201 | -27.59 | 0.37 | 4868850.757 | 743698.8017 |
| 203 | -28.12 | 2.01 | 4868932.676 | 743809.1191 |
| 205 | -28.49 | -0.32 | 4869264.062 | 752645.8982 |
| 207 | -28.69 | -0.09 | 4870315.712 | 752833.2257 |
| 209 | -26.74 | 2.39 | 4870148.85 | 752862.104 |
| 211 | -27.28 | -0.03 | 4869909.534 | 752683.1604 |
| 213 | -28.47 | 1.8 | 4869749.183 | 752707.7679 |
| 215 | -27.3 | 2.81 | 4869875.294 | 752776.9025 |
| 217 | -27.63 | 3.55 | 4870029.842 | 752891.5667 |
| 219 | -29.18 | 2 | 4869884.471 | 752987.9478 |
| 221 | -27.86 | -0.72 | 4869492.414 | 753003.733 |
| 223 | -26.74 | 1.11 | 4870313.845 | 753046.2867 |
| 225 | -26.42 | 0.52 | 4868666.804 | 743958.7699 |
| 227 | -27.15 | 0.16 | 4868931.88 | 743998.8393 |

| Plot Id | $\delta^{13}C$ | $\delta^{15}N$ | UTM Northing | UTM Easting |
|---|---|---|---|---|
| 229 | -27.5 | 1.1 | 4869127.35 | 744081.6572 |
| 231 | -27.06 | 3.4 | 4868667.183 | 744150.0626 |
| 233 | -27.32 | 0.21 | 4869212.37 | 744245.7022 |
| 235 | -26.2 | -0.02 | 4869140.766 | 744264.4186 |
| 237 | -25.9 | -3.22 | 4868853.606 | 744319.2196 |
| 239 | -26.34 | 0.5 | 4868933.252 | 744698.882 |
| 241 | -26.09 | -3.97 | 4869011.232 | 744792.4538 |
| 244 | -26.98 | -0.13 | 4868952.636 | 744862.1378 |
| 246 | -27.07 | -3.9 | 4868885.737 | 744977.9465 |
| 248 | -26.16 | 0.97 | 4868769.214 | 745105.2401 |
| 250 | -24.65 | 3.79 | 4869113.932 | 745253.2355 |
| 252 | -26.42 | 5.36 | 4868817.923 | 745309.2118 |
| 254 | -28.33 | 6.76 | 4869297.325 | 745069.6183 |
| 256 | -25.84 | 5.75 | 4868989.334 | 741226.9983 |
| 258 | -25.03 | -0.25 | 4868907.694 | 741092.5327 |
| 260 | -25.29 | 2.7 | 4868867.504 | 740905.1163 |
| 262 | -27.21 | 10.87 | 4871125.058 | 730218.9371 |
| 264 | -26.98 | 1.86 | 4871248.773 | 730386.5419 |
| 266 | -26.77 | 6.76 | 4871274.873 | 730497.2935 |
| 268 | -26.92 | 2.02 | 4871053.86 | 730696.9907 |
| 270 | -26.56 | 10.36 | 4870780.948 | 730875.2138 |
| 272 | -26.74 | 9.33 | 4870996.429 | 730963.2952 |
| 274 | -26.14 | 10.18 | 4870816.186 | 731056.3504 |
| 276 | -27.15 | 2.72 | 4870832.43 | 731139.3342 |
| 278 | -25.82 | 8.49 | 4870877.526 | 731220.5062 |
| 280 | -27.01 | 1.76 | 4870657.829 | 731393.6821 |
| 282 | -25.67 | 9.52 | 4874804.76 | 760299.7451 |
| 285 | -27.47 | 6.62 | 4877512.786 | 762973.8438 |
| 287 | -26.78 | 12.96 | 4879532.725 | 765215.8585 |
| 288 | -26.13 | 5.04 | 4869505.652 | 753146.3097 |
| 289 | -25.8 | 4.84 | 4869708.828 | 753281.62 |
| 290 | -25.12 | 2.02 | 4870087.337 | 753289.645 |
| 291 | -26.78 | -0.13 | 4869672.854 | 753475.1089 |
| 292 | -25.86 | 0.92 | 4870287.815 | 753557.6588 |
| 293 | -25.22 | 2.16 | 4869770.509 | 753701.2598 |
| 294 | -25.91 | 2.97 | 4870626.253 | 753797.059 |
| 295 | -26.2 | 15.18 | 4870437.081 | 753911.2091 |
| 296 | -27.21 | 6.16 | 4870749.221 | 753986.8382 |
| 297 | -26.31 | 5.66 | 4870275.799 | 754085.3804 |
| 298 | -25.76 | 2.82 | 4869926.196 | 754106.0333 |
| 299 | -25.03 | 2.64 | 4870525.835 | 754136.8661 |
| 300 | -26.74 | 2.49 | 4870255.217 | 754157.7049 |

| Plot Id | $\delta^{13}C$ | $\delta^{15}N$ | UTM Northing | UTM Easting |
|---|---|---|---|---|
| 301 | -25.82 | -0.58 | 4870735.268 | 754173.8321 |
| 302 | -26.25 | 11.05 | 4870215.598 | 754255.6779 |
| 303 | -25.61 | -1.36 | 4870141.642 | 754270.575 |
| 304 | -25.68 | 6.49 | 4870606.205 | 754288.8978 |
| 305 | -26.11 | 14.39 | 4870153.797 | 754384.2441 |
| 306 | -26.37 | 3.19 | 4870451.473 | 754401.7395 |
| 307 | -25.01 | -0.48 | 4870772.537 | 754477.8066 |
| 308 | -25.73 | 14.96 | 4870813.665 | 754274.4978 |
| 309 | -25.94 | 0.82 | 4869097.415 | 751013.9776 |
| 310 | -26.43 | 12.47 | 4869872.371 | 751093.1078 |
| 311 | -25.53 | 6.33 | 4869025.722 | 751117.982 |
| 312 | -27.24 | 8.82 | 4869193.153 | 751133.3365 |
| 313 | -25.98 | 2.33 | 4869317.602 | 751159.1623 |
| 314 | -25.52 | 9.56 | 4869481.169 | 751189.9332 |
| 315 | -25.95 | 2.73 | 4869651.415 | 751191.5123 |
| 316 | -24.1 | 2.36 | 4869334.965 | 751235.6711 |
| 317 | -25.06 | 3.81 | 4869017.907 | 751263.7782 |
| 318 | -25.6 | 2.03 | 4869025.895 | 751269.1022 |
| 319 | -25.03 | 2.1 | 4869274.061 | 751301.4851 |
| 321 | -24.07 | 4.21 | 4869032.806 | 751333.9531 |
| 323 | -25.15 | -0.12 | 4869800.886 | 751349.0073 |
| 325 | -25.55 | 4.44 | 4869436.235 | 751412.6877 |
| 327 | -27.04 | 2.06 | 4869101.618 | 751447.0971 |
| 329 | -25.95 | 14.24 | 4869022.04 | 751606.8736 |
| 331 | -25.74 | 5.15 | 4869111.045 | 751548.8293 |
| 333 | -25.37 | 8.83 | 4868779.384 | 747620.04 |
| 335 | -25.78 | 12.02 | 4869174.894 | 747637.4031 |
| 337 | -26.38 | 1.85 | 4869245.265 | 747823.6621 |
| 339 | -26.04 | 8.04 | 4868907.67 | 747956.8718 |
| 341 | -25.42 | 0.38 | 4869074.521 | 747986.796 |
| 343 | -26.18 | 3.33 | 4869036.31 | 748035.6523 |
| 345 | -25.99 | 7.87 | 4868882.079 | 748164.4166 |
| 347 | -25.34 | 0.61 | 4869008.617 | 748216.7462 |
| 349 | -24.42 | 7.92 | 4869275.1 | 748648.8454 |
| 351 | -26.88 | 5.73 | 4869390.674 | 748924.2235 |
| 353 | -26.03 | -1.53 | 4869478.474 | 749176.5249 |
| 355 | -27.05 | -0.51 | 4869297.178 | 749383.4953 |
| 356 | -25.81 | 4.25 | 4871707.16 | 757186.9933 |
| 357 | -25.95 | 4.46 | 4872333.852 | 757283.1658 |
| 358 | -27.48 | 4.5 | 4872411.394 | 757360.5046 |
| 359 | -26.52 | 3.16 | 4871834.311 | 757395.8122 |
| 360 | -26.43 | 5.28 | 4871956.773 | 757398.2805 |

| Plot Id | $\delta^{13}C$ | $\delta^{15}N$ | UTM Northing | UTM Easting |
|---|---|---|---|---|
| 361 | -26.23 | 2.06 | 4871767.98 | 757406.4289 |
| 362 | -26.96 | 5.04 | 4872317.067 | 757480.6901 |
| 363 | -25.17 | 6.44 | 4872131.738 | 757692.0131 |
| 364 | -25.08 | 8.82 | 4872182.548 | 757796.9099 |
| 365 | -23.59 | 0.99 | 4872761.631 | 757869.9613 |
| 366 | -25.16 | 0.6 | 4872384.498 | 757869.3959 |
| 367 | -24.61 | 2.84 | 4872352.177 | 757953.4225 |
| 368 | -25.02 | 6.87 | 4872828.777 | 757937.2483 |
| 369 | -26.09 | 0.44 | 4872888.304 | 758008.8486 |
| 370 | -24.56 | 0.87 | 4872404.071 | 758057.4683 |
| 371 | -25.3 | 3.35 | 4872662.689 | 758156.6592 |
| 372 | -23.95 | -0.84 | 4872967.737 | 758191.356 |
| 373 | -26.17 | 1.15 | 4872811.425 | 758290.6649 |
| 374 | -26.22 | 5.8 | 4872655.792 | 758435.753 |
| 375 | -24.97 | 4.88 | 4872793.354 | 758454.4839 |
| 376 | -26.25 | 6.33 | 4872665.066 | 758559.1345 |
| 377 | -26.66 | 3.39 | 4872997.791 | 758590.3206 |
| 378 | -27.63 | 7.88 | 4872676.336 | 758619.7623 |
| 379 | -26.58 | 1.06 | 4873161.499 | 758765.5051 |
| 380 | -25.44 | 10.75 | 4873360.097 | 758837.2764 |
| 381 | -27.05 | 2.14 | 4873173.811 | 758767.4338 |
| 382 | -25.62 | 12.01 | 4872481.075 | 758149.2831 |
| 383 | -27.5 | -1.44 | 4869890.136 | 751502.353 |
| 384 | -25.96 | -0.03 | 4869453.869 | 751584.0352 |
| 385 | -26.1 | 2.74 | 4869928.45 | 751661.6499 |
| 387 | -27.22 | -6.59 | 4870004.372 | 751756.8218 |
| 388 | -26.47 | -2.42 | 4869141.347 | 751760.7007 |
| 389 | -25.87 | 1.84 | 4869677.604 | 751763.6217 |
| 391 | -28.58 | -4.04 | 4869957.86 | 751879.1549 |
| 393 | -25.83 | -4.79 | 4869348.08 | 751873.416 |
| 395 | -27.69 | 4.29 | 4869087.181 | 751886.5555 |
| 397 | -27.82 | -1.63 | 4870013.313 | 751933.308 |
| 399 | -26.42 | -5.8 | 4869294.182 | 751947.8121 |
| 401 | -27.94 | 0.51 | 4869238.533 | 752005.3952 |
| 403 | -25.63 | 2.23 | 4869888.839 | 752052.9845 |
| 405 | -26.83 | -4.01 | 4869830.398 | 752066.4624 |
| 407 | -26.04 | 4.8 | 4869395.36 | 752180.2893 |
| 409 | -25.94 | 8.62 | 4869808.085 | 752269.0619 |
| 411 | -26.51 | -0.53 | 4869423.002 | 752380.1948 |
| 413 | -27.73 | 0.98 | 4868194.733 | 739958.5786 |
| 415 | -27.55 | -2.85 | 4868812.047 | 740021.4094 |
| 417 | -25.64 | -0.94 | 4868768.578 | 740049.5084 |

| Plot Id | $\delta^{13}C$ | $\delta^{15}N$ | UTM Northing | UTM Easting |
|---|---|---|---|---|
| 419 | -26.26 | -0.82 | 4868764.601 | 740062.5124 |
| 421 | -26.48 | -0.45 | 4868282.41 | 740229.4985 |
| 423 | -26.4 | 1.59 | 4868862.365 | 740120.8557 |
| 425 | -25.95 | -0.52 | 4868953.924 | 740253.3648 |
| 427 | -25.22 | 3.03 | 4868902.147 | 740358.9244 |
| 429 | -25.72 | 7.86 | 4868209.211 | 740296.4584 |
| 431 | -23.95 | 3.51 | 4868430.731 | 740393.7178 |
| 433 | -25.9 | 0.69 | 4868461.443 | 740473.7853 |
| 435 | -24.53 | -1.2 | 4868502.299 | 740557.5026 |
| 437 | -24.91 | -0.9 | 4868570.017 | 740645.8694 |
| 439 | -27.27 | 1.99 | 4868782.772 | 740777.1872 |
| 441 | -26.25 | 3.53 | 4870059.264 | 754558.2797 |
| 443 | -26.34 | 10.1 | 4870114.832 | 754585.8829 |
| 446 | -27.39 | 6.03 | 4870152.608 | 754815.9186 |
| 448 | -27.87 | 4.42 | 4870353.074 | 754937.6071 |
| 450 | -27.54 | 6.43 | 4870405.168 | 755077.0613 |
| 452 | -26.2 | 1.84 | 4870974.795 | 755108.153 |
| 454 | -25.83 | 4.36 | 4871137.398 | 755142.0685 |
| 456 | -27.02 | 2.3 | 4870313.607 | 755183.4717 |
| 458 | -28 | 10.29 | 4871214.139 | 755199.3842 |
| 460 | -24.71 | 6.15 | 4870820.115 | 755279.6788 |
| 462 | -26.96 | 10.32 | 4871238.657 | 755344.7042 |
| 464 | -25.99 | 4.48 | 4871297.278 | 755681.5839 |
| 466 | -26.24 | 5.16 | 4871234.344 | 755722.5913 |
| 468 | -27.49 | 13.67 | 4871174.021 | 755974.8645 |
| 501 | -27.79 | 2.95 | 4868478.369 | 740449.861 |
| 502 | -27.69 | 3.44 | 4868424.326 | 740431.7284 |
| 503 | -26.84 | 3.23 | 4868468.994 | 740375.4496 |
| 504 | -26.4 | 2.28 | 4872436.8 | 758097.9764 |
| 505 | -26.18 | 10.92 | 4869033.654 | 741405.4188 |
| 506 | -28 | 10.45 | 4868967.243 | 741383.7277 |
| 507 | -26.45 | 3.85 | 4869822.172 | 750472.8987 |
| 509 | -25.58 | 10.16 | 4871532.701 | 755797.2351 |
| 510 | -26.52 | 6.15 | 4871549.176 | 755737.1686 |
| 511 | -27.16 | 7.55 | 4872766.325 | 757819.1572 |
| 512 | -27.42 | 12.41 | 4872898.597 | 757987.5562 |
| 513 | -26.71 | 13.33 | 4874595.642 | 760022.8954 |
| 514 | -28.28 | 6.9 | 4874631.313 | 760047.9064 |